Activity and Expression of Na/K-ATPase in Thoracic Aorta of Normal Pregnant and Experimental Preeclamptic Rats

by

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ACTIVITÉ ET EXPRESSION DE LA NA/K-ATPASE DANS L'AORTE DE RATE AVEC PREÉCLAMPSIE EXPÉRIMENTALE

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RÉSUMÉ

La gestation chez le rat s'accompagne d'une diminution des effets des vasoconstricteurs, laquelle est absente chez la rate avec prééclampsie expérimentale (PE), un désordre induit durant la gestation et caractérisé par de l'hypertension et un état augmenté de vasoconstriction. Ces manifestations sont considérées être associées aux changements de l'activité de Na/K-ATPase (pompe à sodium) dans le muscle lisse vasculaire, qui contribue elle à l'entretien d'un gradient électrochimique de Na^+ et de K^+ à travers la membrane cellulaires. Cependant, elle joue un rôle important dans le reglage du tonnees vasculaire. Les études précédentes ont prouvé que des ligands endogènes de la pompe à sodium sont augmentés dans le plasma et utérine pendant la gestation et encore plus en PE. Dans ce travail, nous proposons que l'activité et l'expression de la pompe à sodium sont modifiées dans le muscle lisse vasculaire durant la gestation et à la PE. Nous avons mesuré les effets inhibiteurs de la ouabaïne sur l'activité de la pompe et l'expression protéique de la sous unité α de la Na/K-ATPase dans l'aorte de rate gestante avec ou sans PE. Pour développer le modèle de preeclamptie expérimentale, nous soumettrons des rates gestantes à un supplément sodique pour 7 jours (saline 0.9% , comme breuvage), correspondant à la dernière des 3 semaines de gestation chez cette espèce. Des anneaux d'aorte des rates sont suspendus dans des bains à organe et des réponses au KCl sont obtenues en l'absence ou en présence de ouabaïne. L'activité de la Na/K-ATPase est mesurée indirectement par la relaxation au KCl dans une solution physiologique sans K^+. L'expression de la sous-unité α1 est mesurée par buvardage Western. La ouabaïne produit des contractions sur les anneaux d'aorte, cette réponse est augmentée dans une solution faible en K^+, de même que chez la non-gestante sous diète forte en sel. Le groupe gestant s'est montré résistant aux effets vasoconstricteurs du KCl, mais pas les rates avec prééclamptie expérimentale. La relaxation au KCl est réduite dans l'aorte de rate gestante comparaterement à la non-gestante. La ouabaïne inhibe cette relaxation, avec de façon plus importante durant la gestation. L'expression de la sous-unité α1 de la Na/K-ATPase est réduite dans les aortes de rate gestante et augmentée dans la prééclampsie. L'ensemble de nos résultats suggèrent que l'activité de la Na/K-ATPase est augmentée dans les muscles lisses vasculaires durant la gestation pour favoriser les l'hyperpolarisation des cellules musculaires lisses et

diminuant ainsi leurs réponses aux vasoconstricteurs. Le PE diminue l'effet inhibiteur de l'ouabaïne, ce qui suggère que l'activité de pompe à sodium serait réduite dans cette condition. L'expression augmentée de la protéine de la sous-unité α1 bourrait être un effet compensatoire à cette réduction d'activité.

Mots Clés : Prééclampsie, Na/K-ATPase, supplément sodique, ouabaïne, aorte, muscles lisses vasculaires, vasoconstriction

ABSTRACT

During late gestation in rats, resistance to vascular responses to vasopressor agents is observed, but absent in rats with experimental preeclampsia (PE), a pregnancy-induced disorder characterized by high blood pressure and increased vasoconstriction. These manifestations are believed to be associated with changes in the activity of Na/K-ATPase (sodium pump) in vascular smooth muscle, since it contributes to the maintenance of an electrochemical gradient of Na^+ and K^+ across cell membrane and plays a major role in vascular tone regulation. Previous studies showed that endogenous sodium pumps ligands are increased during pregnancy and even more in PE. In the present work, we hypothesize that sodium pump activity and expression in vascular smooth muscle would change in response to pregnancy and PE. Thoracic aortas of normal pregnant and of experimental preeclamptic rats were assayed for inhibitory effects of ligands of the sodium pump, as well as for protein expression of α-isoform of Na/K-ATPase. Non-pregnant and pregnant rats were used; experimental preeclamptic model was developed by giving pregnant rats 0.9% NaCl supplement to drink for the last 7 days of gestation. Responses of aortic rings to KCl were obtained in the presence of ouabain, and inhibitor of sodium pump, in normal (5.8 mM) or low (2.0 mM) potassium medium. Relaxations to KCl were obtained by incubating aortic rings in K^+-free solution and precontracting with phenylephrine (PhE) in the presence of inhibitors of the sodium pump. KCl was gradually re-added into the bath to induce relaxation. Protein expressions of α1 and α3 isoforms of sodium pump were measured by Western blot. It was found that Ouabain produced contractions on isolated aortic rings, which was increased in low K^+ solution, as well as in non-pregnant rats with high salt diet. Pregnant group showed refractoriness to vasoconstrictor effects of KCl, but experimental preeclamptic rats not. Relaxation to KCl was significantly reduced in aorta from pregnant compared to non-pregnant rats. Ouabain produced concentration-dependent inhibition in this relaxation, that was significantly larger in aorta of normal pregnant than in non-pregnant rats. Expression of α1 subunit of sodium pump did not change in aortas of pregnant rats, but was significantly increased in aorta of preeclamptic rats. Based on the above observations and experimental results, we conclude that sodium pump in vascular smooth muscle is increased during pregnancy to favor vascular smooth muscle

hyperporization and thus reduce vasoconstriction. Experimental PE decreased the inhibitory effect of ouabain, which suggests sodium pump activity is decreased in pathological state of PE. Increased protein expression of α1 isoform may be a biological compensational effect.

Key words: Preeclampsia, Na/K-ATPase, sodium supplement, ouabain, aorta, vascular smooth muscle, vasoconstriction

This thesis is dedicated to my family for their love, support and encouragement.

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LIST OF ABBREVIATIONS

ANG II	angiotensin II
ADP	Adenosine diphosphate
ANP	atrial natriuetic peptide
AVP	arginine vasopressin
ATP	Adenosine triphosphate
DMSO	dimethylsulfoxide
EDLF	endogenous digitalis-like factor
E_{max}	maximal response
EC_{50}	concentration producing 50% of maximal response
ECL	enhanced chemiluminescence
HRP	horseradish peroxidase
KBS	Krebs bicarbonate solution
MBG	marinobufagenin
Na/K-ATPase	sodium pump, sodium-potassium ATPase
NO	nitric oxide
NP	non-pregnant
NP-40	Nonidet P-40
PE	preeclampsia
Pg	pregnant
PhE	phenylephrine

OLC	ouabain-like compound
RAAS	rennin-angiotensin-aldosterone system
SDS	sodium dodecyl sulfate
SPL	sodium pump ligand
SVR	systemic vascular resistance
TBS	tris base solution
VDCC	voltage-dependent calcium channels

1. INTRODUCTION

1.1 Pregnancy

In human being, normal pregnancy causes profound physiologic changes in several systems to adapt to a new situation with different challenges and pregnancy needs. These maternal changes concern cardiovascular, hematological, pulmonary, urinary and endocrine systems, etc. For example, there is a large expansion of plasma volume, an increase in heart rate, cardiac output, all components of renin-angiotensin-aldosterone (RAAS) system, and elevated salt retention. All these body changes are made to accommodate the demands of gestation.

1.1.1 Vascular Functions in Pregnancy

During normal pregnancy, dramatic changes take place in cardiovascular system, such as: increased heart size (by about 12%), increased cardiac output by (30-40%), increase heart rate by about 15% beats/ min, increase renal blood flow (by about 70%) and increase blood volume (by 50%), decrease peripheral vascular resistance and blood pressure [123].

Despite the large increases in blood volume and cardiac output (Fig.1) [37], arterial blood pressure does not rise during normal pregnancy. In contrast, the diastolic blood pressure decreases in mid-pregnancy and then gradually increases after 26 to 28 weeks to pre-pregnancy values at term (Fig.2) [38]. The decreased blood pressure results from decreased systemic vascular resistance (SVR). The SVR decreases to a minimum at midpregnancy followed by a gradual rise until term [1].

Moreover, plasma renin activity, plasma angiotensin II (ANG II) and aldosterone level increase 5 to 10 times during this period. However, the normal pregnant women also have some refractoriness to the pressor effects of exogenously given ANGII. It has been shown

that nulliparous women who later become preeclamptic lose this refractoriness prior to developing clinical signs of PE [2].

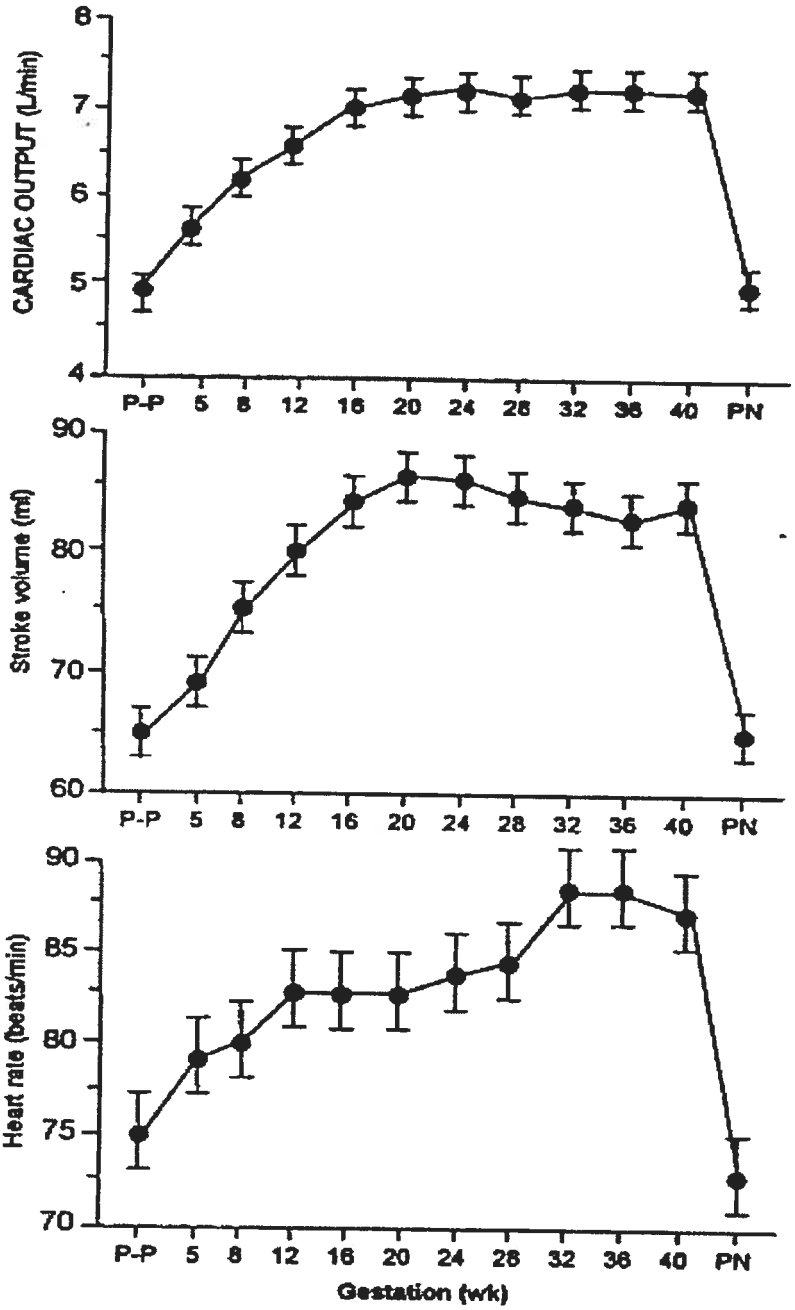


Figure 1. Changes in cardiac output (upper panel), stroke volume (middle panel), and heart rate (lower panel) throughout pregnancy in human. P-P, pre-pregnancy values; PN, values after parturition. (From [37] Hunter S, Robson S: Br Heart J 68: 540, 1992)

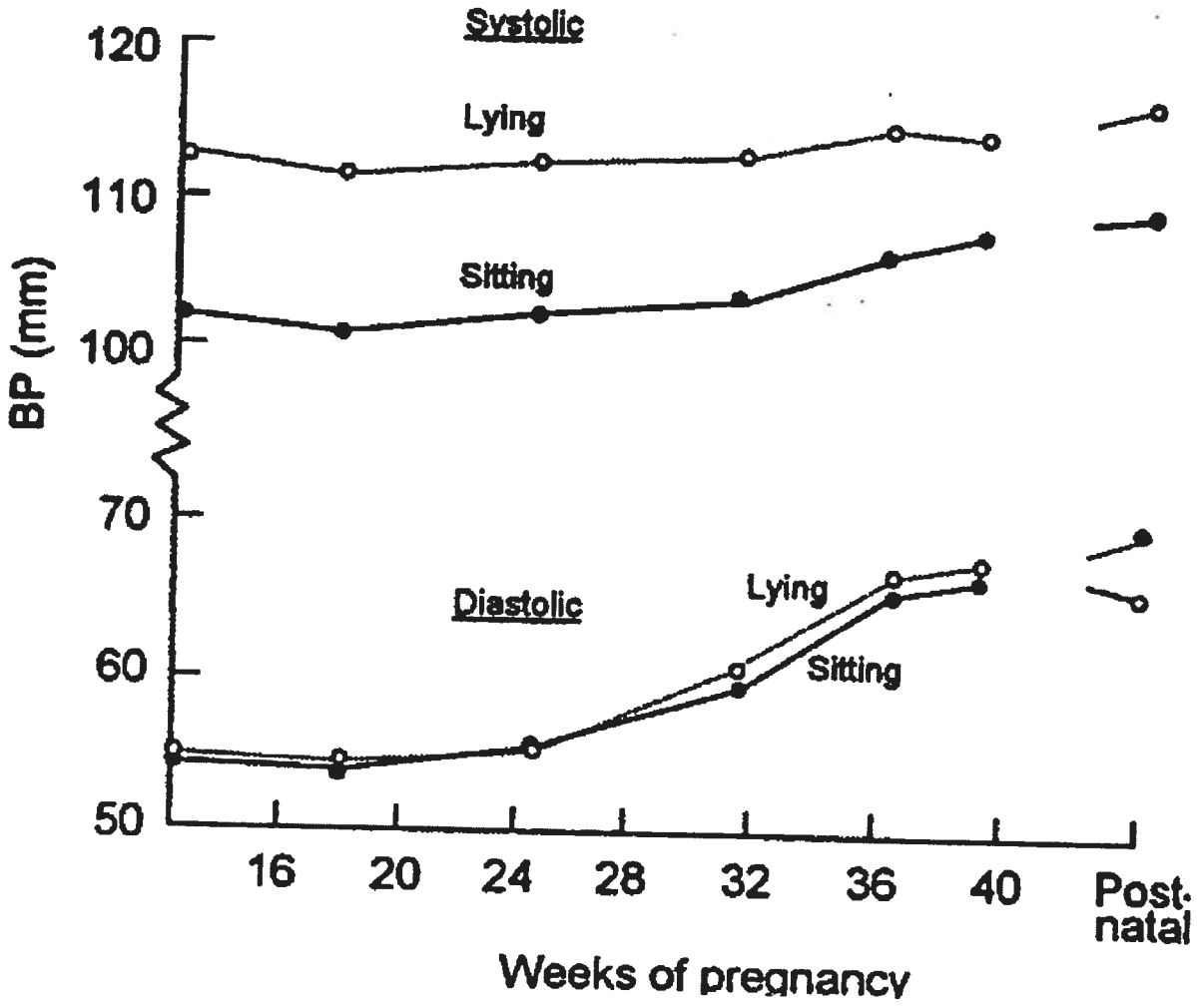


Figure 2. Blood pressure profiles (sitting and lying) during pregnancy. (From [38] MacGillivray I, Rose G, Rose B: Clin Sci 37: 395, 1969)

1.1.2 Mechanisms for Pregnancy-Induced Decrease in Blood Pressure

As mentioned, the decrease in blood pressure is attributable to the fall in decreased SVR, which is believed to link to diminished constriction of blood vessels [79]. The exact mechanisms for the fall in SVR during pregnancy are poorly understood. Several theories have been proposed for this hemodynamic adaptation, such as down-regulation of membrane receptors for vasopressor ligands (vasopressin, norepinephrine, etc.) in vascular tissues, increased liberation of an endogenous vasodilator (prostacyclin, nitric oxide, etc.) acting as physiological antagonist to vasopressor [66]. However, pregnancy-associated blunted responses to vasoconstrictors has been observed in isolated blood vessels of pregnant rats in the presence and absence of endothelium, indicating that the phenomenon is not consequent to autonomous reflex or to some endothelial dysfunction [65, 76-79]. More recent hypothesis proposes smooth muscle-relaxing effects of the elevated progesterone level, the presence of the one or more circulating substances exerting a vasodilatory effect on the arterial and venous vasculature. This may involve nitric oxide (NO), prostaglandins or atrial natriuretic peptide (ANP) [1]. Attention has focused on the production of NO in pregnancy, since it plays a role in the control of vascular resistance in human [76]. However, no single mechanism emerges as the only and major one.

1.2 Pharmaco-mechanical Coupling, the Na/K-ATPase

1.2.1 Membrane Hyperpolarization in Pregnancy

A promising explanation for the decreased vascular resistance is membrane hyperpolarization in vascular smooth muscle cells. Electrophysiological data have shown that resting membrane potential is more negative in vascular smooth muscle cell membrane of vasculature during pregnancy in rat [80]. This pregnancy-induced hyperpolarization is believed to relate to alteration in cellular active transport system across the plasma membrane, change in activity of Na/K-ATPase (also known as the

sodium pump). This transport system is believed to play an important role in maintaining membrane potential. Increased sodium pump activity may promote hyperpolarization in plasma membrane, and reductions of its activity can result in increase muscle contraction [67]. Studies of blood cells from patients with essential hypertension have generally demonstrated reductions in Na/K-ATPase activity [68]. In the present study, we investigate the activity and protein expression of the Na/K-ATPase in aortic rings of rats to attempt to determine its contribution to pregnancy-induced decrease in blood pressure.

1.2.2 Biological Functions of Na/K-ATPase

The sodium pump is responsible for establishing and maintaining the electrochemical gradient in animal cells. For every molecule of ATP hydrolyzed, three Na ions from the intracellular space are extruded and two K ions from the external medium are uptaken (Fig.3). Thus, the sodium pump contributes substantially to the maintenance of cellular ionic homeostasis. The electrochemical gradients are then harnessed by other membrane proteins for a variety of essential cellular functions, including electrical membrane potential changes mediated by ion channels, osmotic balance of the cell, active uptake of molecules, like neurotransmitters, amino acids, sugars, nucleosides, and extrusion of Ca^{2+}. It is also thought to be critically involved in functions such as cellular growth and differentiation, as well as contraction of vascular smooth muscle. Therefore the inhibition of the Na^+ pump produces an ionic redistribution that may promote the increase in vascular tone [3].

1.2.3 Subunit Compositions of Na/K-ATPase

The Na/K-ATPase is a member of a large family of P-type ATPase, which harness the energy derived from the hydrolysis of the terminal pyrophosphate bond of ATP to drive the transport of cations such as Na^+, K^+, H^+, Ca^{2+}. It is composed of 3 protein subunits (α, β, γ).

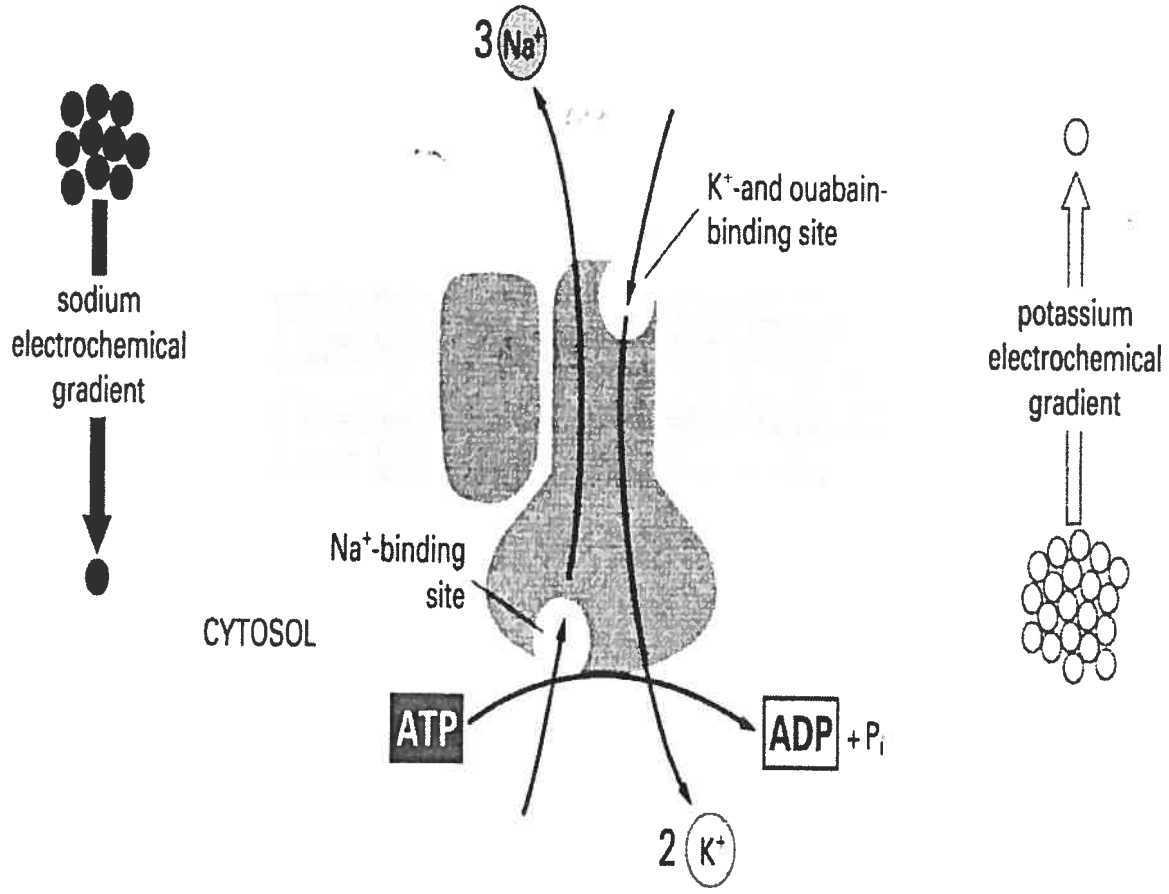


Figure 3. The sodium pump. This carrier protein actively pumps Na^+ out and K^+ into a cell against their electrochemical gradients. For every molecule of ATP hydrolyzed, three Na^+ are pumped out and two K^+ are pumped in. The specific inhibitor ouabain and K^+ compete for the same site on the extracellular side of the pump. (From [119] Bruce Alberts et al: Molecular Biology of The Cell, Fourth Edition, Chapter 11.)

The α subunit, which is referred to as the catalytic subunit, is responsible for most of the activities. It contains also intracellular ATP hydrolytic site and the extracellular digitalis glycoside-binding site. It has a relative mass of 100-113 KDa, depending on the isoforms α1 to α4. α subunit crosses the membrane 10 times, forming trans-membrane domains M1 to M10; both N- and C- termini are localized on the cytosolic side (Fig.4).

The β subunit is highly glycosylated and has a relative molecular mass of about 60 KDa. The mass of the protein moiety of this unit is 36-38 KDa, depending on the isofors β1- β3. The β subunit crosses the membrane only once, and the N-terminus is localized on the intracellular side of trans-membrane (Fig.4). The proper roles of these proteins are still not entirely clear. More recent results have shown that the β subunit makes direct contact with the α subunit [4], thereby stabilizing the α subunit and assisting in its transport from the endoplasmic reticulum to the plasma membrane [5].

The third subunit of Na/K-ATPase, the γ subunit of 7-11 KDa, was first identified as a component involves in the binding of [^{3}H] ouabain, but γ subunit expression is not seen in all tissues where α, β expression is otherwise easily identified. Like the β subunit, the γ subunit spans the plasma membrane once but unlike the β subunit, its amino terminus is extracellular. A putative 3-dimensional model of the α, β and γ subunits of the Na/K-ATPase is shown in figure 4 [39].

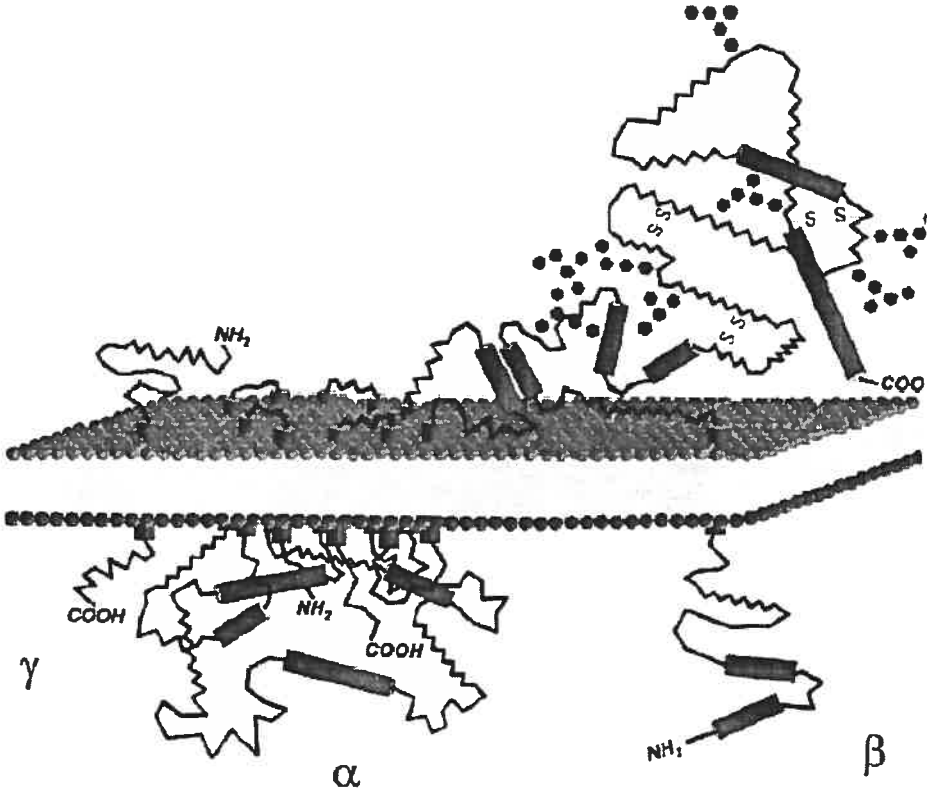


Figure 4. Putative three-dimensional model of the topological structure of the Na/K-ATPase (From [120] Mobasheri A et al: Bioscience Reports. Vol. 20, N0.2: 55, 2000)

1.2.4 Distribution and Physiological Function of Na/K- ATPase

Thus far 4α, 3β and 1γ isoform subunit have been identified in mammals [6, 9]. Each isoform has a unique tissue distribution and is encoded by a separate gene, and may be altered in pregnancy or pathologic processes, such as hypertension [6]. The α1 polypeptides are ubiquitously distributed in animal cells whereas the remaining α isoforms exhibit a more restricted tissue specific and developmental pattern of expression. The α2 isoform is expressed most abundantly in cardiac muscle, skeletal muscle, adipose tissue and glial cells in the brain [10, 11], while the α3 isoform is found in high concentrations in neurons [10, 11] and cardiac muscle [12, 10]. The α4 isoform appears to be specific to the testis [7].

Expression of β1 subunit polypeptides has been detected in many tissues from which Na/K-ATPaes has been isolated, such as brain, heart and kidney [24]. In contrast, the β2 isoform is more tissue-specific. It appears to function as an adhesion molecule on glial cells specifically involved in mediating interactions between neurons and glia [13]. The β3 isoform is the most recently described member of the β isoform gene family and is expressed predominantly in the testis but also in the brain, kidney, lung, liver, etc. [10, 12]. The function of the β subunit has yet to be elucidated.

Although the enzyme is a hetero-dimer consisting of at least one α and β subunit, studies suggested that the kinetic properties are largely dictated by the catalytic α isoform. The diversity of Na/K-ATPase subunit isoforms and their complex spatial and temporal patterns of cellular expression suggest that Na/K-ATPase isozymes perform specialized physiological function. It is expected that their expression and/or function are altered in pathogenic states or conversely that alterations in their expression and/or function contribute to the disease.

1.2.5 Function of Na/K-ATPase in Vascular Tissues

The vascular sodium pump structure is similar to that found in other tissues, but with some particularities. The presence of these small structural differences in the ATPase may permit variations in the responses and regulation of sodium pump function between vascular smooth muscle and other tissues [17]. The enzyme of vascular tissue has been difficult to isolate and purify using conventional protein purification techniques, due to low number of copies of the pump in these cells [14]. Nevertheless, the importance of the Na^+ gradient in blood pressure regulation has long been recognized, and many studies have been carried out to characterize the enzyme and its role in modulating vascular contraction [14-16]. Studies on the regulation of its activity have shown that the vascular enzyme can be stimulated by treatments that increase intracellular Na^+ concentration and/or decrease intracellular K^+ concentration [18]. In the present study, low K^+ solution was applied to increase the activity of the sodium pump and thus the inhibitory effect of ouabain, a potent blocker of sodium pump. More recent studies have shown that the increase in intracellular Na^+ by mechanical strain [19, 20] or chronic treatment with ouabain [19], up-regulate α1- and α2- isoforms expression of the sodium pump in aortic smooth muscle cells.

Na/K-ATPase plays an essential role in the control of vascular smooth muscle tone. Thus, the inhibition of this enzyme by cardiac glycosides or K^+-free solution produces an increase in vascular tension in several vessels [21, 22]. The accepted cellular mechanism involved in the contraction after the Na pump blockade is that an increase in intracellular Na^+ concentration causes an increasing Ca^{2+} entry into cells through the inversion of Na/Ca exchange [23, 25]. The Na/Ca exchanger is a transporter present in the plasma membrane catalyzing the counter-transport of three Na^+ for one Ca^{2+} and helps to maintain steady Ca^{2+} balance. It is primarily a Ca^{2+} extrusion mechanism, under normal physiological conditions. When the sodium pump is inhibited, internal Na^+ rises, promoting reverse exchange (Na^+ goes out and Ca^{2+} comes into the cell), leading to an elevated internal Ca^{2+}, and thus the contraction.

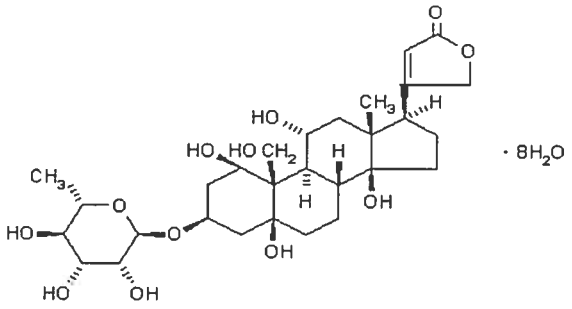
1.2.6 Specific Inhibitors of Na/K-ATPase

The Na/K-ATPase is specifically inhibited by a series of naturally occurring steroids, termed cardiac glycosides, such as ouabain and digitalis (Fig.5). Cardiac steroids compete with K^+ and bind reversibly to an extracellular side of the Na/K-ATPase (Fig.3) and inhibit ATP hydrolysis and thus ion transport. K^+ lowers the affinity of the enzyme for cardiac steroids at their high affinity extracellular binging site. Cardiac steroids, especially water-soluble ouabain, have often been used to identify Na/K-ATPase and to study ion transport mechanisms involved in this system. Inhibition of the sodium pump by cardiac steroids has some clinical use. Application of these substances, especially of digitalis and its congeners, helps to increase muscular contractility of the failing heart, possibly by indirectly inducing an elevation in the Ca^{2+} concentration in the myocardium.

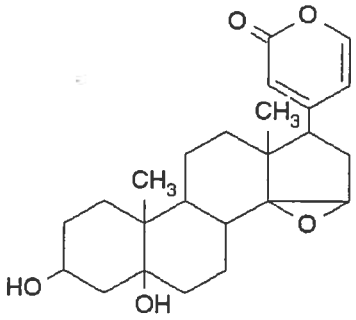
As described above, the α-subunit is the specific receptor site for digitalis glycoside-like inhibitors and is represented by three well defined functional isoforms (α1, α2 and α3). They are distributed in a tissue-specific fashion; exhibit differential sensitivity to the inhibitory ligands [70]. For example, ouabain displays high affinity to α3 isoform (predominant in nerve endings) while α1 isoform (predominant in vascular smooth muscle) is more sensitive to inhibition by marinobufagenin (MBG) [70]. Besides, studies in the rat showed a consecutive inhibition of the α2 and α1 isoforms by MBG with high and low affinity, respectively [71].

1.2.7 Endogenous Digitalis-Like Factors

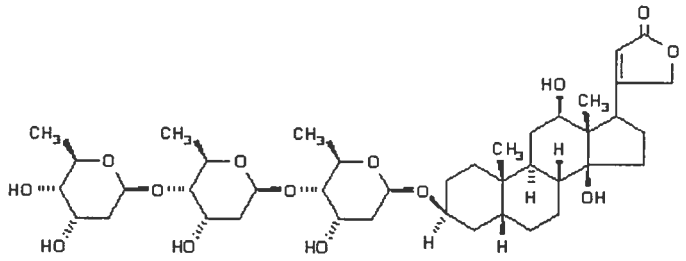
Since cardiac glycosides were suggested as endogenous physiological regulations of heart muscle contraction [61], there has been a long search for natural endogenous ligands of the sodium pump and many studies have then looked at involvement in maintenance of high blood pressure in hypertension. Mammalian plasma was found contain several substances that inhibit the sodium pump and interact with digitalis antibodies [28, 32]. Ouabain-like factor (OLF) was the first mammalian endogenous digitalis-like factor (EDLF) to be purified [31]. Figure 6 shows the structure of ouabain and its analogs [99].



ouabain



marinobufagenin



digoxin

Figure 5. Structure of three cardiac glycosides

But recent evidence suggests that at least one of the mammalian EDLFs has a bufodienolide structure [33-35]. Bufodienolides are cardioactive steroids that were initially described in amphibian, and differ from cardenolides in having a doubly unsaturated six-membered lactone ring at the C17 position of the steroid nucleus. It has been shown that human plasma and urine contain material that cross-reacts with antibodies against one of the toad-derived bufodienolides, marinobufagenin [33] (see Fig.5).

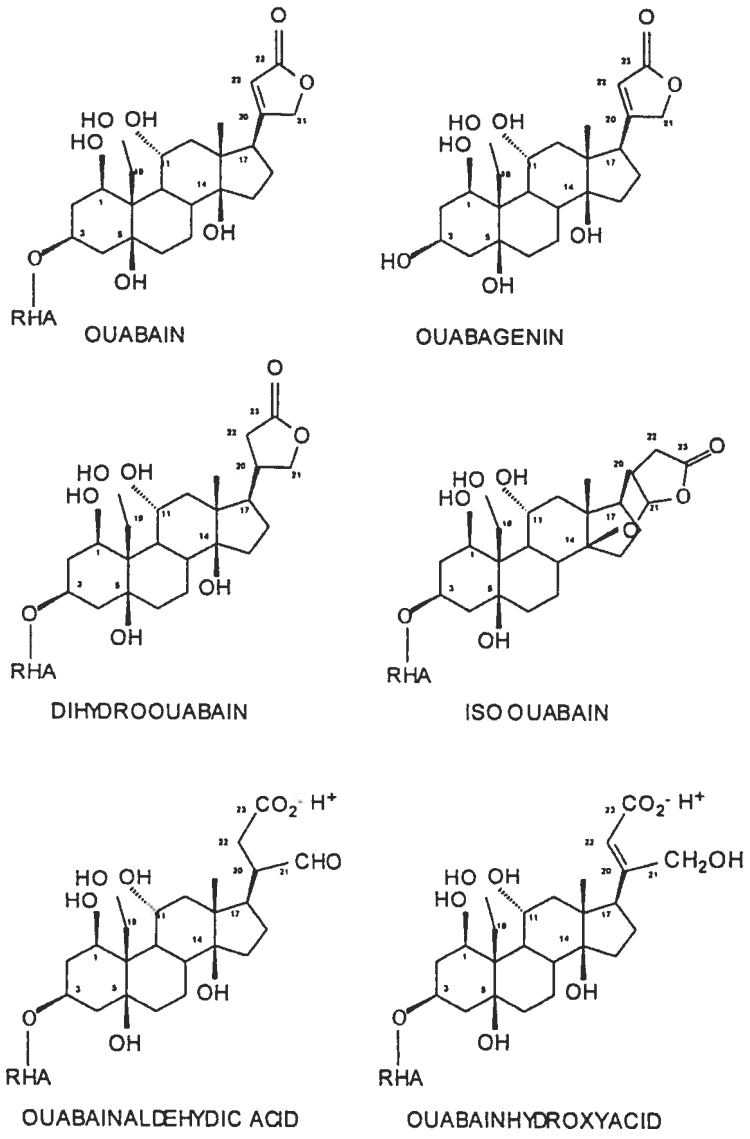


Figure 6. Structure of the ouabain analogs (From [99] Manunta P, Hamilton BP, Hamlyn JM: *Hypertension* 37 (part 2): 472, 2001.)

Elevated amount of EDLFs has been found in hypertensive animals and humans [26, 27]. They are thought to contribute to the pathogenesis of hypertension [3, 29]. Expansion of plasma volume is known to stimulate endogenous cardiac glycosides, e.g. EDLFs [30]. It

has been hypothesized that in volume-expanded hypertension, cardiac glycosides are stimulated to promote natriuresis via inhibition of the sodium pump in renal tubules [3]. However, excessive production of EDLFs may also inhibit the sodium pump in vascular smooth muscle and result in vasoconstriction. Therefore, sodium pump inhibition would induce increased natriuresis and would protect blood volume at the expense of an elevated blood pressure [3, 72].

Late pregnancy is indicated to be associated with plasma volume expansion involving renal sodium and fluid retention [113]. It has been demonstrated increased level of cardiac glycosides in pregnancy and hypothesized that they are involved in pathogenesis of pregnancy-induced hypertension [114, 115]. Subsequent studies demonstrated increased level of EDLFs in normotensive pregnancy [116-118] and in PE [69, 115, 117, 118]. Table 1 shows the EDLF values in plasma from control women vs women with normal pregnancy and in PE [69].

Evidences that an elevated plasma cardiac steroids may raise the blood pressure is that increased plasma cardiac steroid levels were correlated with the height of blood pressure in volume-expanded hypertension [122], including pregnancy-induced hypertension [114], the administration of affinity-purified ovine digoxin antibody FAB Fragments lowers the blood pressure in PE [121], and that antibody to MBG lowers blood pressure in pregnant rats on a high NaCl intake [133]. However, putative role of EDLFs in pregnancy and PE is still controversial and there is little information regarding the change in sodium pump activity accompanying volume-expansion caused by elevated amount of endogenous sodium pump ligands during end gestation and in PE. Recently published data by Fedorova et al is the first report that administration of an anti-MBG antibody to NaCl-loaded pregnant rats reduces blood pressure and increases vascular smooth muscle sodium pump activity [133].

Table 1. Plasma levels of marinobufagenine (MBG) and ouabain-like compound (OLC) in non-pregnant control individuals, the third trimester of normal pregnant women and patients with PE. (From [69] Lopatin DA et al: Journal of Hypertension 17: 1179-1187, 1999)

	MBG (nM)	OLC (nM)
Non-pregnant control	0.19 ± 0.04 (11)	0.297 ± 0.037 (11)
Normal pregnancy	0.625 ± 0.067 (6)	0.32 ± 0.07 (6)
Preeclampsia	2.63 ± 0.10 (15)	0.697 ± 0.16 (15)

1.3 Preeclampsia

1.3.1 Definition of Preeclampsia

Preeclampsia is a complex pregnancy disorder characterized by hypertension and proteinuria, developing after 20 weeks gestational age. Hypertension refers to blood pressure of 160 mm Hg or greater systolic or 110 mm Hg or greater diastolic, recorded on at least two occasions at least 6 hours apart with patient at bed rest. Proteinuria is defined as the urinary excretion of 0.5 g protein or more in a 24-hour specimen. High blood pressure in PE is mainly due to a reversal of the vasodilation characteristic of normal pregnancy, replaced by marked increases in peripheral resistance [92].

PE is associated with substantial risks. For the fetus, these include intrauterine growth restriction, death, and prematurity with inherent morbidity, whereas the mother is at risk for eclampsia, renal failure, pulmonary edema, stroke, and death. PE is unique to human pregnancy, and there are still no clinically useful screening tests to identify women in whom it will develop. Antihypertensive therapy lowers maternal blood pressure but does not improve fetal outcomes; the only "cure" is the delivery of the infant.

Compared to normal pregnancy, PE is associated with maternal hypertension, decreased circulatory volume, and reduced activation of RAAS [36]. It has been observed that the vascular reactivity to vasopressor ANGII is greatly enhanced in PE compared with that of normal pregnancy [2]. Other manifestations such as an increase in maternal vascular tone, enhanced platelet aggregation, and reduced uteroplacental blood flow are observed [75]. Understanding the mechanisms producing these changes in hemodynamics and vasculature in PE will give strong clues to the overall pathogenesis of this disorder.

1.3.2 Causes of Preeclampsia

Preeclampsia remains a leading cause of maternal and fetal morbidity and mortality worldwide. Despite extensive research, the mechanisms that cause PE are still debated. At present, 4 hypotheses are intensively investigated: inadequate placental implantation, the existence of a factor X released from the placenta into the maternal blood causing endothelial cell damage [73], immune maladaptation, and genetic predisposition.

The placenta is considered the pathogenic focus for all manifestations of PE, because delivery is the only definitive cure for this disease. Defective placentation and placental vascular insufficiency are the starting point of PE. Early in normal gestation, trophoblast cells invade placental bed, leading to remodeling of the spiral arteries into maximally dilated low resistance vascular channels, unable to constrict to vasoactive stimuli [93, 94], thereby guaranteeing a high flow volume to the uteroplacental bed (see Fig.7) [73]. In women who eventually develop PE, the invasion of the uterine spiral arteries is incomplete, with the vessels remaining thick-walled and muscular [95], and with failure of endothelium-dependent relaxation [96]. The cytotrophoblasts in preeclamptic women seem to fail to express vascular-type adhesion molecules, which impair their ability to form sufficient connections with the uterine vessels [94, 97, 98].

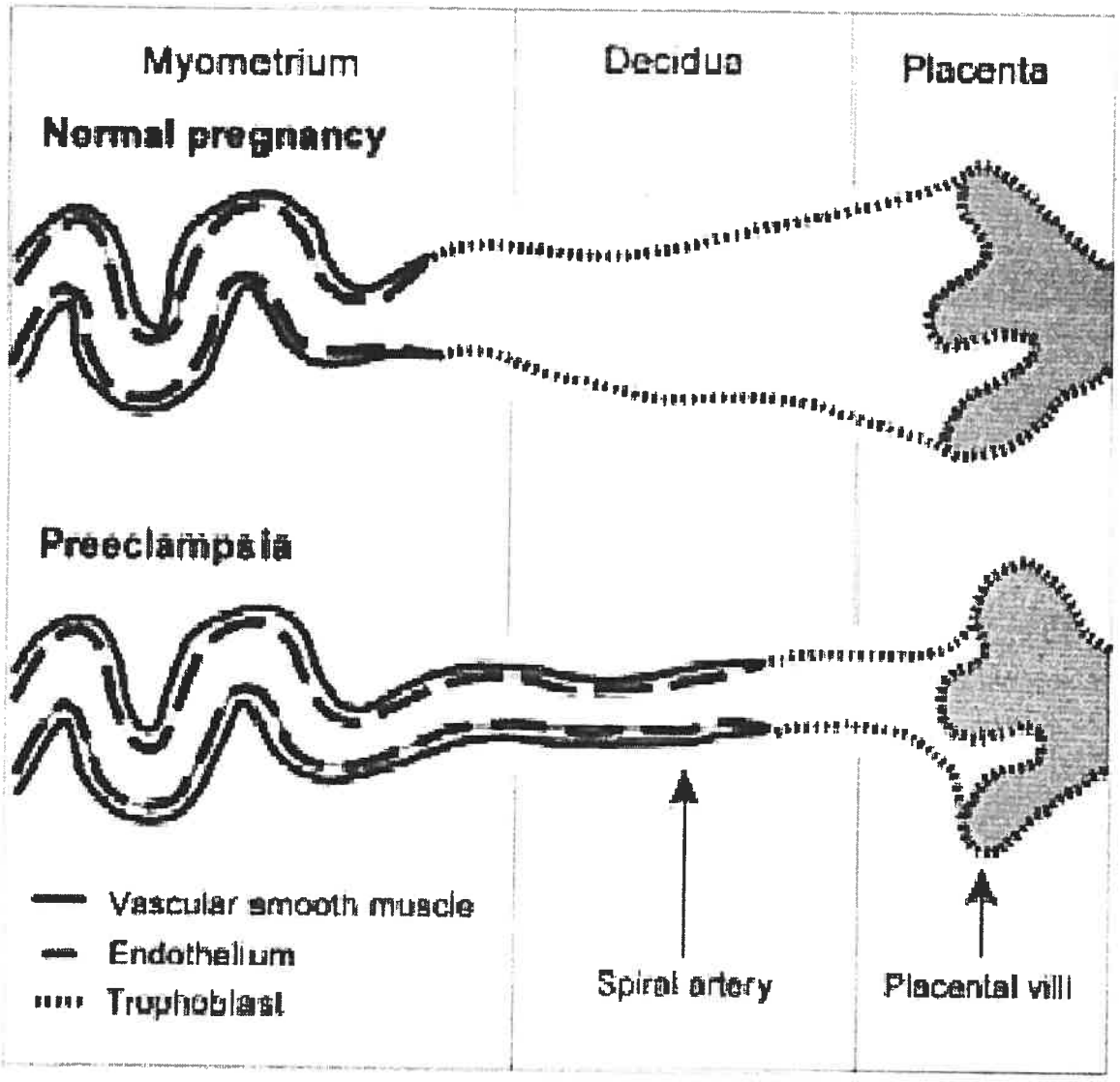


Figure 7. Trophobalst invasion into the spiral arteries in the placental bed in normal pregnancy and in preeclampsia. (From [73] VanWijk MJ et al: Cardiovascular Research 47: 39, 2000.)

The endothelium, a monolayer of epithelial cells, is in direct contact with the blood and constitutes a physical and metabolic barrier. According to the wide range of functions displayed by endothelial cells, it is an attractive hypothesis that endothelial cell dysfunction plays an important role in the pathophysiology of PE. To link the placental

abnormalities to the generalized endothelial dysfunction seen in PE, the existence of a factor X, released from the placenta into the maternal circulation, was proposed [73]. The disturbed placentation supposedly leads to hypoperfusion of the placenta and ischemia, resulting in the release of one or more unidentified factors from the placenta. Factor X then causes the late vascular dysfunction of PE, consisting mainly of generalized endothelial dysfunction, resulting in vasoconstriction, activation of the coagulation system and redistribution of fluids, the symptoms of PE, and often in fetal growth restriction [73].

Another hypothesis about the etiology of PE postulates that abnormal placentation, with failure of the trophoblast to induce physiological dilation and remodeling of spiral arteries, is caused by a maternal immunological response to foreign paternal antigens of the fetoplacental unit [100]. This theory is supported by the greater incidence of PE among multiparous women becoming pregnant with a new partner. Lymphocytes of preeclamptic subjects do not show the cellular hyporesponsiveness to fetal cells that is typical of normal pregnancy [101], the activity of circulating natural killer cells, neutrophils and cytokines, such as TNF-α, IL-6, IL-2 and IL-12 is increased [100, 102]. Besides, in PE HLA-G, a surrogate auto-antigen known to prevent recognition by natural killer cells is not expressed as general in the placenta as in normal pregnancy [103]. The resulting activation of leukocytes in the deciduas, can cause release of cytokines, elastase and oxygen free radicals, all of which cal interact with endothelial function.

A familial factor in the pathogenesis of PE has been recognized for many years, but the exact mode of inheritance and the interaction between maternal and fetal genotypes are still under discussion. Several studies have shown an increased frequency of PE in the sisters, daughters and granddaughters, but not the daughters-in-low, of women who have had the disease [104-106]. These data have been interpreted to mean that the putative susceptibility genes act through the mother. More recently it was suggested that PE is a polygenic trait [107]. Implicated in this process are the angiotensin gene, the endothelial nitric oxide synthase gene, and genes involved in TNF-α-production, thrombophilic disorder, hypertension and obesity [108-112]. Thus far most studies investigating the role

of genetics in PE have been small scale and a large database of genotypes, present n women with PE and their children, is needed to elucidate to which extent genetics are involved in PE.

1.4 Experimental Model of Preeclampsia

PE has been a recurrent research area for many years. The multitude of systems involved in the physiopathology of PE and their complex interactions provide an intriguing challenge. A major problem in PE research is the lack of a valid animal model that can enable a more thorough investigation of physiopathology this disease. Over the past decade, various mammalian models have been proposed to study the pathogenesis implicated in PE, including:

1. Inhibition of nitric oxide (NO) synthesis [40]
2. Reduced of the uterine perfusion [41]
3. Transfecting animals with genes of human renin-angiotensin system [46]
4. Stimulating the sympathetic nerve [47]
5. Administration of endothelin-1 during late pregnancy [48]
6. Administration of low doses of endotoxin [49]

Although providing valuable information on relevant mechanisms, these models are not specific to the pregnant condition and occasionally are associated with fetal mortality [42]. Thus, since PE is a pregnancy-specific disease, none of these approaches would represent an adequate animal model.

An ideal animal model of PE should fulfill some criteria:

- It should exhibit increase in arterial pressure similar to that occurring in PE.
- It should occur only in pregnant animal.
- It should manifest many of the physiological characters of PE.
- The PE-condition induced should be relieved by delivery of pups.
- It should be feasible in small animals, ideally.
- It should be simple to perform and reproducible.

1.4.1 High Sodium Intake Model

Sodium supplementation during gestation can provide an excellent animal model to study the mechanisms implicated in PE. It's been demonstrated that high sodium intake (0.9 % NaCl) to the rats at the end of gestation, the last 7 days of the 22-days pregnancy in this species, prevented the decrease of blood pressure and reversed the diminish of vascular reactivity normally observed in this period [42,74] (Fig.8). In women, blood pressure already decreased by the end of the first trimester and returned to pre-gestational values approaching term [43]. In pregnant rats, blood pressure does not change until the seventeenth and eighteenth days and then gradually decreases until term (23rd days) [44]. Thus, the cardiovascular changes observed in the last week of pregnancy in rats correspond to the ones occurring during the second trimester in pregnant women [42, 74]. Women who subsequently develop PE at the end of gestation commonly do not show the characteristic decrease in blood pressure at the second trimester.

As shown in Figure 8, before sodium supplementation (day 12 to day14), systolic blood pressure was similar in both groups of pregnant rats. Sodium supplement 0.9% did not affect systolic blood pressure in non-pregnant rats. However, pregnant rats receiving 0.9% NaCl supplement did not show the expected decrease of blood pressure observed in control pregnant rats [42]. It was also observed that sodium supplement of 0.9% NaCl decreased the activity of renin-angiotensin-aldosterone system (RAAS) in non-pregnant and pregnant rats. However, the decreased RAAS induced by high salt intake was not associated with any change in systolic blood pressure in non-pregnant rats [45], but with suppression of the decrease in blood pressure in pregnancy. This result suggests that the mechanisms controlling blood pressure are easily perturbed by high-sodium intake during pregnancy [42].

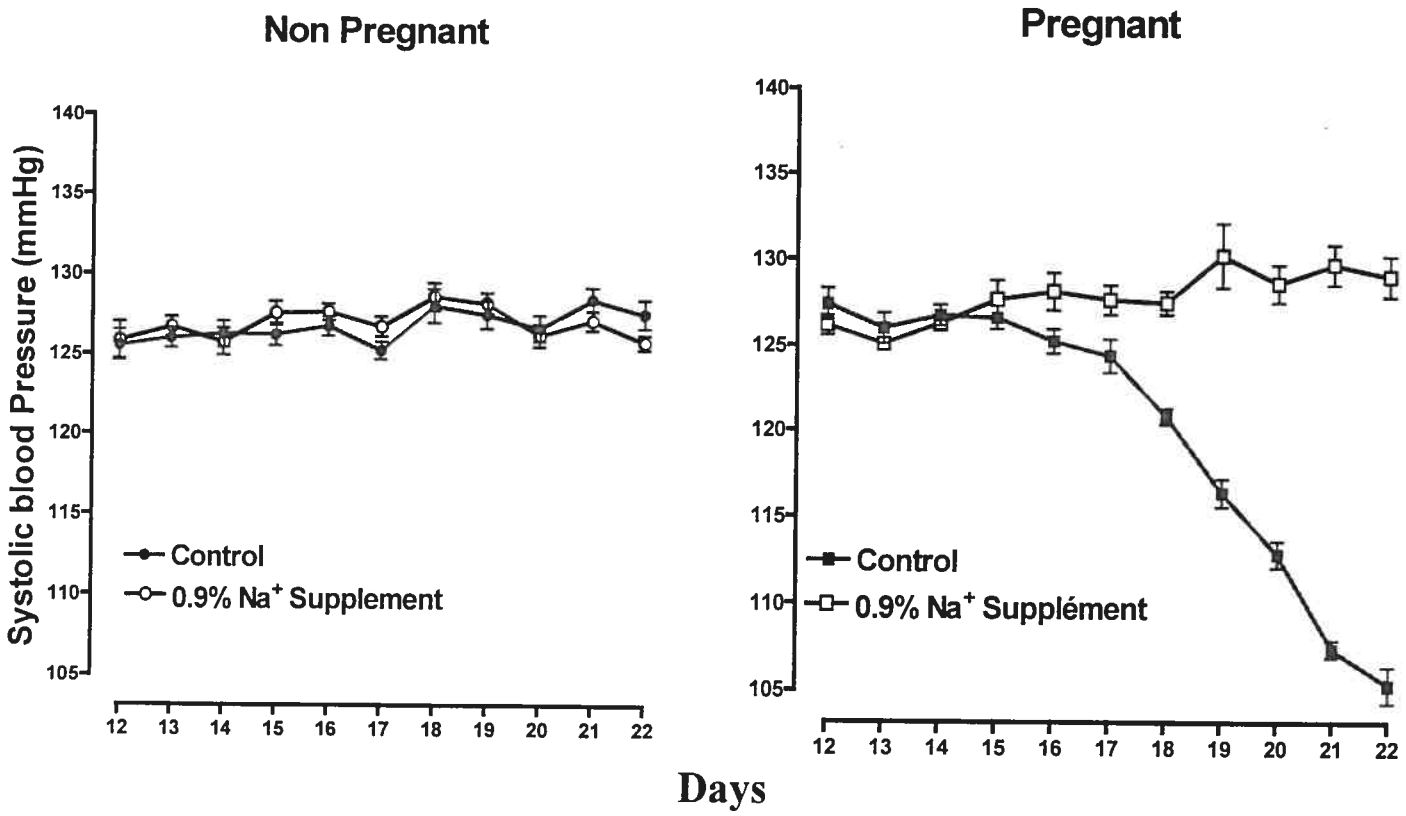


Figure 8. Arterial pressure of the rats in normal diet or in diet with sodium supplement (Adapted from [42] Beausejour et al: A.J.P. 285: H375, 2003)

In a parallel study, contractile response to vasoconstrictors (phenylephrine, KCl, arginine vasopressin) in the aortic rings of normal pregnant and pregnant rats on high sodium intake have also been measured, and increased responses to these vasoconstrictors in aortic rings of pregnant rats on 0.9% sodium supplement compared with pregnant rats on normal water was observed [74]. This demonstrated that augmented sodium intake during gestation in the rat is linked with the reversal of gestational-associated resistance to vasopressors and indicates that this is an experimental model showing some features of gestational hypertension [74].

1.4.2 Stress in Pregnancy Model

Stress in pregnancy rats was here proposed as animal model for human PE [51]. This model is based on epidemiological data on human populations, showing that stress has unfavorable effects on pregnancy and results in a greater incidence of PE and intrauterine growth retardation [52, 53]. In establishing this experimental model, the rats were exposed to some sound stimulus associated with overpopulation in cages between days 7 and 14 over 22 of pregnancy. Pregnant female Wister rats experienced more manifestations from intense stress than did the non-pregnant animals, such as high arterial blood pressure, increased proteinuria, higher adrenal weight, lower endothelium-derived relaxing factor activity, decreased weight gain, lower fetal weight and greater number of fetuses. The alterations found in the rats were similar to those occurring in human PE [51].

As we know, PE occurs spontaneously only in the human species. Humans are the most intellectually endowed living beings and lead a complicated life in society in which behavioral rules control most primitive emotional reactions. Thus, in some individuals, many aspects of daily routine may lead to intense chronic stress. Given these considerations, Nitol et al believe that intense stress during pregnancy may provoke or favor PE [51].

1.4.3 Other Model: Adaptive Transfer of Activated Th1 Cells

Experimentally induced PE on the basis of immunological imbalances associated with endothelial cell dysfunction has also been reported. This immunological approach proposed that the immune system is important in the course of pregnancy and that it possibly participates in the etiology of PE [50]. This animal model was developed by transferring activated BALB/c Th1-like splenocytes into allogeneically pregnant BALB/c female mice during late gestation (on days 10 and 12 of pregnancy). The authors hypothesized that activated Th1 cells will negatively affect late allogeneic and induce PE-like symptoms [50]. The model mimicked the symptoms of PE, i.e. increased blood

pressure and glomerulonephritis accompanied by proteinuria, which were not detectable in non-pregnant recipients of activated Th1-like cells. Adoptive cell transfer adversely affected the outcome of pregnancy by increasing fetal rejection, with uterine immune cells showing an inflammatory profile. This excessive maternal inflammatory response leads to generalized endothelial cell dysfunction [50].

The study results showed that adoptive transfer of Th1-like cells induces an increased cytokine production (TNF- α and IL-12) and Th1 marker (CCR5) in uterine lymphocytes. Th1 cytokines can directly damage organs and destroy vessels [57], which is believed to be induced by direct interaction of immune cells (secreting Th1 cytokines) with the vessels [50]. Before this, it's also been reported that Th1 cells play a detrimental role during mammalian pregnancy [54], [55]. And there is a close relationship between activated Th1 immune cells and PE in humans [56].

How the transfer of activated Th1-like cells provokes these physiological abnormalities exclusively in pregnant animals? It is believed that since the transferred cells produced predominantly Th1-type cytokines, they expand and stimulate host cells primed to paternal antigens toward the secretion of inflammatory cytokines [58]. Increased secretion of Th1 cytokines by activated host cells or, in other words, an inflammatory host response, was in fact proposed to be the main cause leading to PE in humans [59], [60].

In summary, both of the latter two animal models, to some degree, may present some features and physiological changes that resemble those observed in PE. But since PE is mainly characterized by maternal hypertension, which is resulted from enhanced vessel constriction, and is associated with enhanced vascular reactivity [38] and reduced activity of RAAS [36], only the first model – 0.9% NaCl supplementation, can represent these altered hemodynamic parameters similar to those occurring in PE, and thus make a valuable tool to study the mechanism implicated in PE [74].

2. Research Objectives

The evidence that endogenous sodium pump ligands (SPL)/endogenous digitalis-like factors are increased during pregnancy and even more in PE [69,133] suggests that alteration in activity and expression of vascular sodium pump may contribute to these special physiological or pathological states. The purpose of this work is to investigate the inhibitory effects of ligands of the sodium pump and protein expression of α subunit of the Na/K-ATPase in isolated aorta of rats. The objectives are approached by the following ways:

1. Evaluate the contractile effect of the inhibition on the Na/K-ATPase with ouabain in thoracic aorta of normal pregnant and experimental preeclamptic rats in normal physiological solution or in reduced potassium medium. We investigated the inhibitory effect of ouabain as the first research object because it is a well-known compound and the first discovered cardiac steroid that selectively and potently blocks the plasmalemmal sodium pump in a variety of cell types. Ouabain like compound was the first mammalian endogenous digitals-like factor to be purified [31]. The rat vascular sodium pump is very resistant to inhibition by cardiac glycosides ouabain, therefore high concentrations of this compound (0.1-1mM) were needed to observe a clear response [124-126].

2. Measure the inhibitory activity on KCl-induced relaxation by three sodium pump ligands (ouabain, MBG and digoxin) in a K^+-free solution. The concentrations used of MBG (0.01-0.1μM) were based on the physiological concentration. Digoxin worked as a reference and similar concentrations (0.1-1.0 μM) were applied.

3. Measure the protein expression of α-subunit of Na/K-ATPase in the aorta of normal pregnant and experimental preeclamptic rats.

3. Materials and Methods

3.1 Animal

Female Sprague-Dawley rats (Charles River Canada, St-Constant, PQ, Canada) aged 10-11 weeks were mated with age-matched males. The morning on which vaginal smears were found to contain spermatozoa was labeled Day1 of pregnancy. The pregnant females were then placed in individual caged until used on the 22nd day of gestation. Virgin rats of the same age served as controls. All the animals were fed a normal diet containing 0.23% NaCl. Control animals (pregnant and non-pregnant) had tap water during all the treatment period. The experimental group received 0.9% NaCl solution as a beverage for 7 days, starting on day 15 of experimentation, corresponding to the last week of gestation, these are called experimental preeclamptic rats. At the end of treatment (day 22 of gestation), the animals were decapitated, and thoracic aorta were collected rapidly and placed in cold krebs bicarbonate solution (KBS) or stored at -80°C for western blot analysis.

3.2 Materials

Chemicals were purchased from Fisher Scientific (Montreal, PQ, Canada). All chemicals were of analytical grade. Ouabain, Digoxin, Dimethlsulfoxide (DMSO), Cocktail Protease Inhibitors and Hepes were purchased from Sigma Chemical Co. (St. Louis, MO), NP-40 Alternative was from Calbiochem. Marinobufagenin (MBG) was kindly provided by Dr. Bagrov (NIH/NIA, Baltimore, MD). Phenylephrine hydrochloride, Carbacol were obtained from Research Biochemical International. Anti-Na/K-ATPase α1, α3 subunit antibodies were purchased from Upstage Biotechnology. Mouse monoclonal [AC-15] to β-actin-loading control was from Novus Biologicals. Anti-mouse HRP-linked second antibody, molecular weight markers and ECL Western blot detection solution were purchased from Amersham Biosciences. The BioRad protein assay kit was from BioRad (Hercules,CA).

3.3 Organ Bath Assay

Isolated thoracic aorta was cleaned of fat and connective tissues and cut into consecutive rings of 3-4 mm. The rings were mounted on stainless steel hooks and placed in individual jacketed tissue baths (10 ml; Radnotti Glass, Monrovia, CA) maintained at 37 (± 0.5) °C and oxygenated. In order to exclusively study the regulation of Na pump in smooth muscle of the vessels, the vascular endothelium was removed by gently rubbing with injection needle.

The aortic rings were equilibrated for 60 min under 2.0 g passive tension with frequent washing and tension adjustment. The tissues were bathed in KBS (PH 7.4) of the following composition: 118 mM NaCl, 4.65 mM KCl, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.18 mM $MgSO_4$, 1.18 mM KH_2PO_4, 5.5 mM dextrose. Normal KBS contained 5.83 mM of K^+, with reduced K^+-KBS had 2.0 mM K^+. Tension was measured by isometric force-displacement transducers (FT-03; Grass Instruments, Quincy, MA) and recorded on a computerized data acquisition system using Work Bench software (Kent Scientific, Litchfield, CT). Figure 9 illustrates the experiment of organ bath assays.

To confirm the removal of endothelium, after equilibration, the tissues were challenged with 1.0 μM phenylephrine (PhE). At plateau responses, Carbacol (10 μM) was added. There should be no relaxation present. If any relaxation was observed, the endothelium was further rubbed. The experiments with ouabain were performed under a sodium lamp to prevent photo-degradation of this substance.

3.4 Concentration – Response Curves to KCl

To evaluate the contractile effect of ouabain on sodium pump, concentration – response curves to KCl were measured in the presence and absence of ouabain in normal physiological solution ($[K^+]$ 5.8mM) or in reduced potassium medium ($[K^+]$ 2.0mM). Eight aortic rings from each of non-pregnant, pregnant or experimental preeclamptic rats were used and divided into two groups; four rings were incubated in normal KBS and the

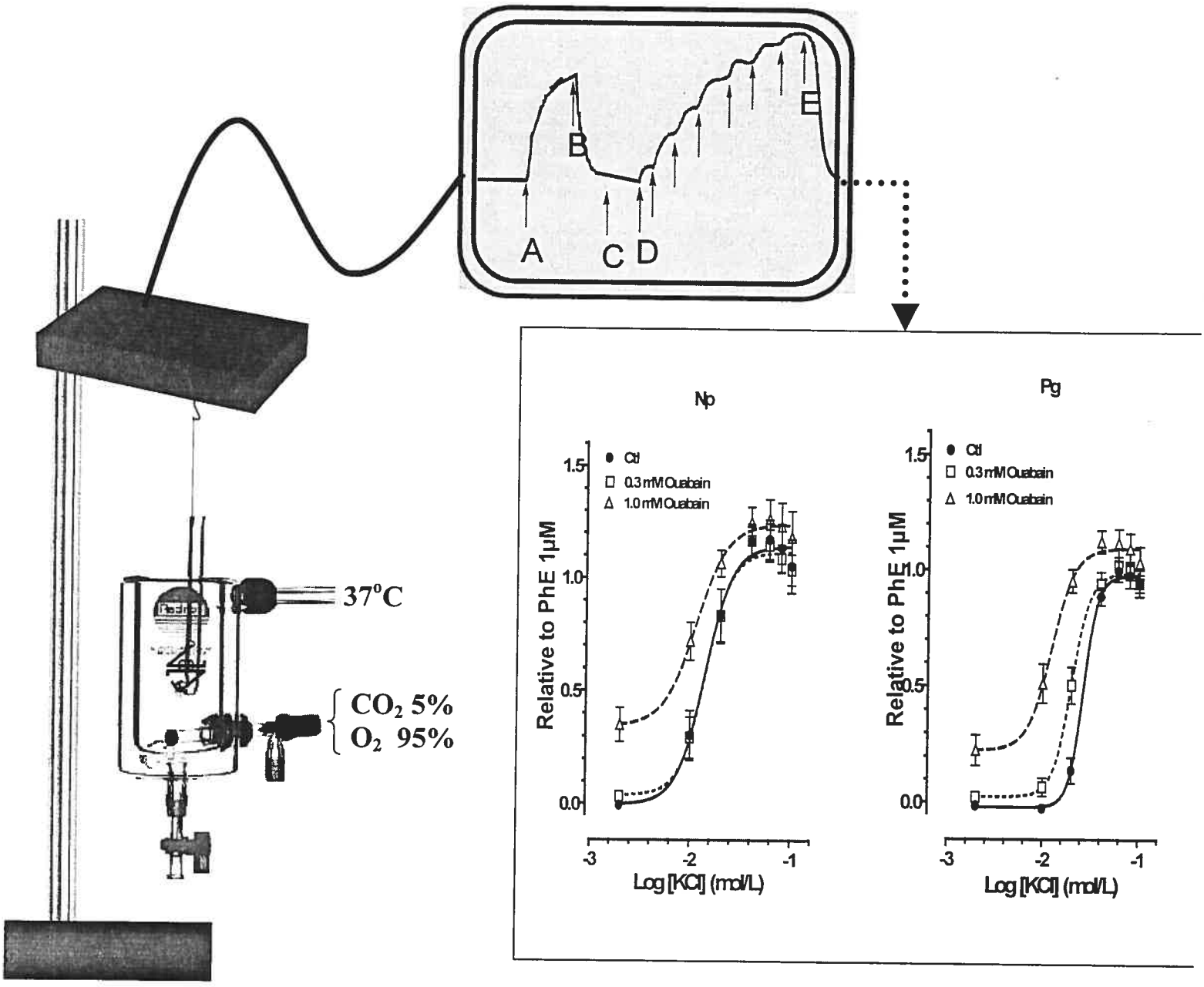


Figure 9. Organ bath assays

other four were bathed in reduced potassium KBS. After initial response to PhE and Carbacol, the solution of half the rats were changed to low potassium KBS. After 60 min stabilization period, one ring of each group served as control while the other three were challenged with increasing concentrations of ouabain (0.1, 0.3, 1.0 mM). Fifteen min later, KCl (2.0 mM to 100 mM) was cumulatively added to the baths (Complete protocol see Fig.10). Figure 11 shows a typical experimental record of KCl-induced concentration.

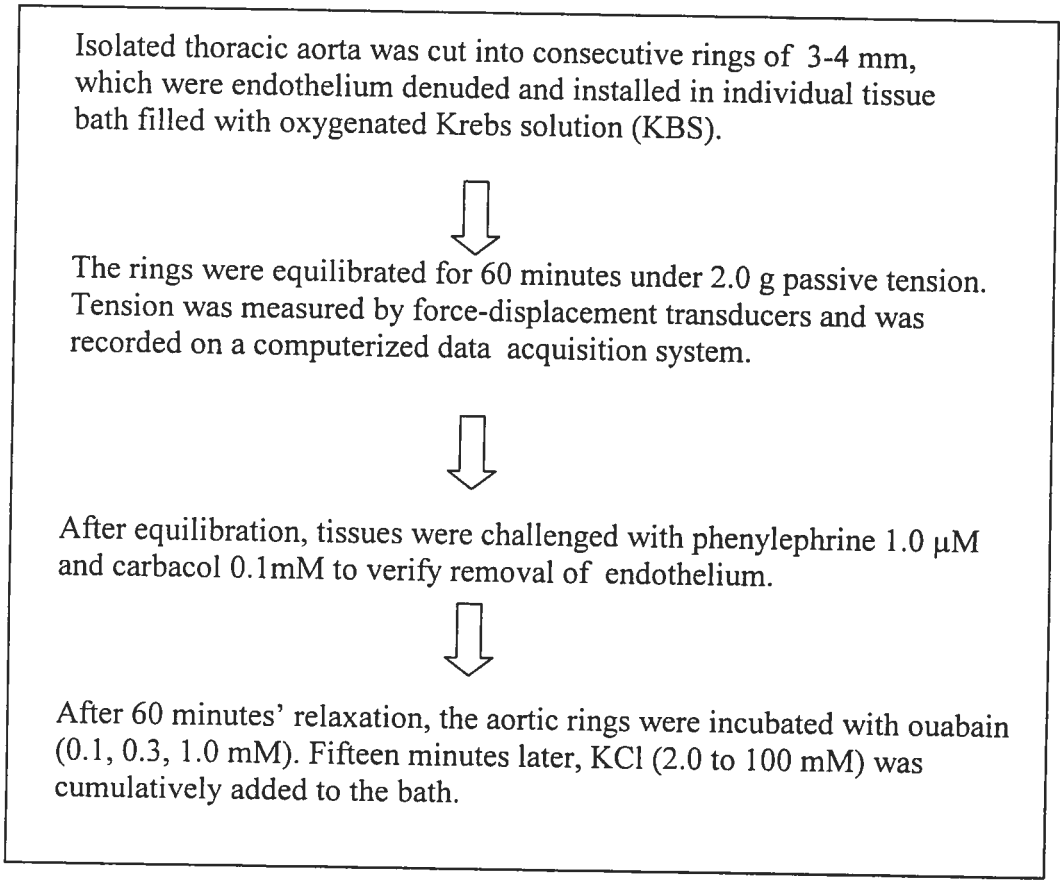


Figure 10. Experiment protocol of KCl-induced contraction

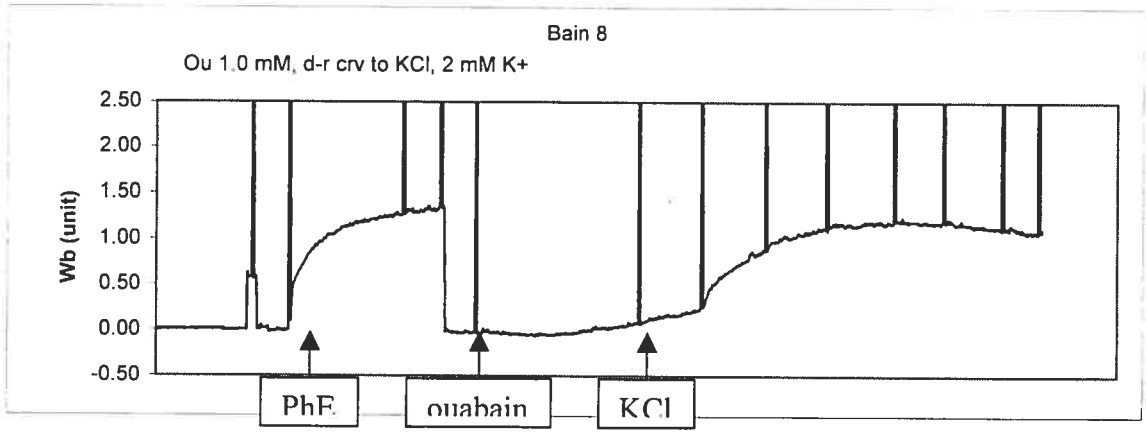


Figure 11. Experiment record of KCl-induced contraction

3.5 Relaxation Curves to KCl

To further measure the inhibitory activity of Na pump ligands, another indicator of the functional activity of the Na/K-ATPase, K^+- induced relaxation in a K^+- free environment was conducted in aortic rings of pregnant and non – pregnant rats. After equilibration, all eight rings were exposed to K^+- free solution. The K^+ - free buffer solution (PH 7.4) was prepared by substituting KH_2PO_4 and KCl with NaH_2PO_4 and NaCl, respectively, on an equimolar basis. Then, one ring used as control, three were treated with different concentrations of ouabain: 0.01, 0.03, 0.1 mM, two were treated with digoxin 1 μM and 0.1 μM and 2 tissues with marinobufagenin (MBG) 0.1 μM and 0.01 μM. After 5 min, they were made to contract with PhE 1.0 μM. At plateau response, KCl was gradually re–added into the tissue bath in a cumulated fashion (0.1-6.0 mM) to induce a relaxation curve. The relaxation response after each addition of KCl reached a steady state within 3-5 min. In the present experiment, Phenylephrine (1μM) was used for two times. The first application was to conform the removal of endothelium, and the second one was to produce a contraction before the KCl-induced relaxation. (Complete experimental protocol see Fig.12). Figure 13 shows a typical record of KCl-induced relaxation.

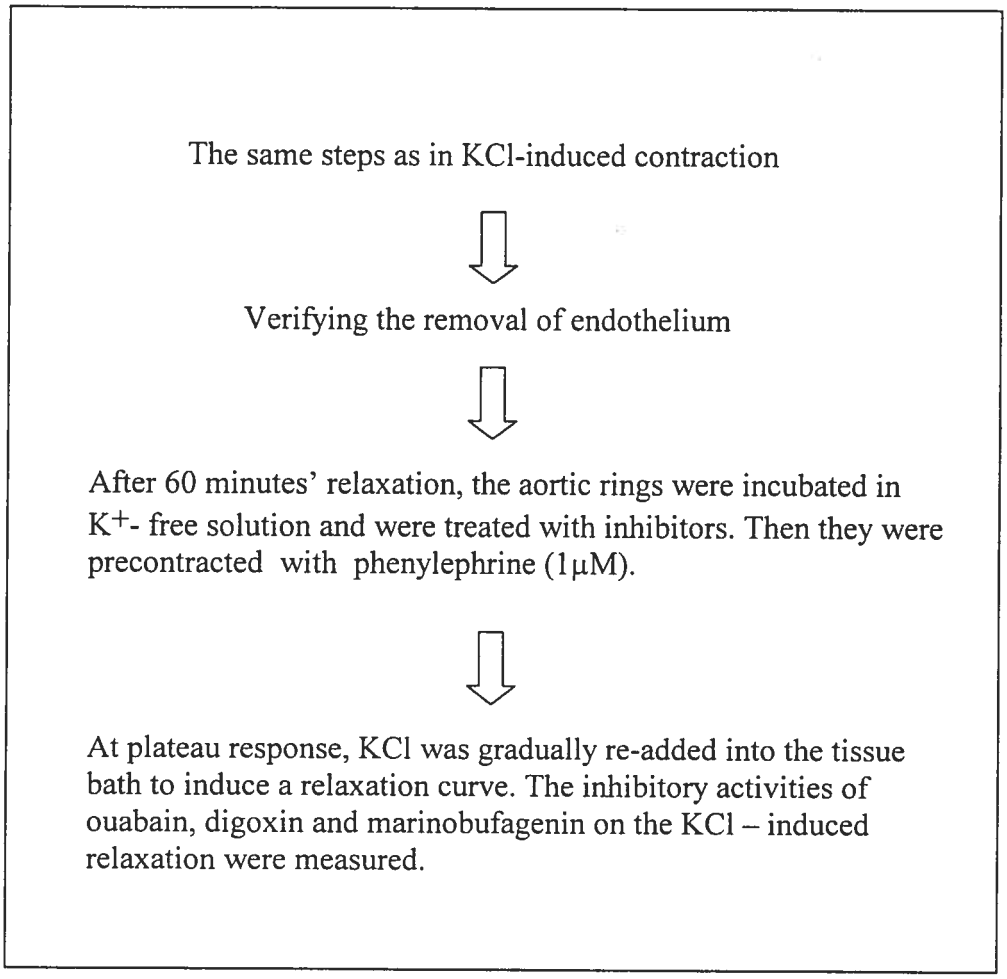


Figure 12. Experiment protocol of KCl-induced relaxation

3.6 Tissue Protein Preparation

Five aortas of non-pregnant, pregnant or experimental PE rats were collected, frozen and powdered in liquid nitrogen. Tissue were homogenized in ice-cold buffer (Hepes 20 mM, NP-40 Alternative 1%, PH 7.4) containing 10% cocktail protease inhibitors (Table 2). Nonidet P-40 is a nonionic mild detergent which can solubilize the target proteins in an immunoreactive and undegrated form. The homogenates were centrifuged at 3000 rpm (Hermle Z360K) for 10 min at 4°C to get rid of the nuclei and debris. The supernatant fraction was recovered and the protein concentration was measured by the Bradfrod method (BioRad). Then the quantified protein samples were mixed with electrophoresis

indicator Laemmli to make a final protein concentration of 1.5 mM. The extracts were kept ice cold during this experiment and were stored in -20°C for further use.

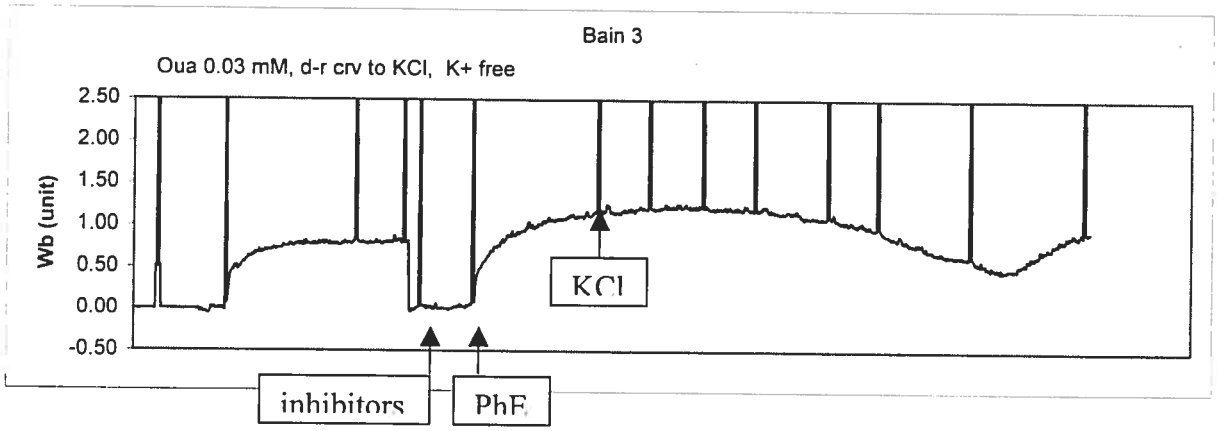


Figure 13. Experiment record of KCl-induced relaxation

Table 2. Cocktail Protease Inhibitors

Inhibitors	Concentration (mM)	Proteases
AE BSF	2	Saine
ED+A	1	Metallo
Bestatin	0.13	Amino peptodases
E-64	0.014	Cysteines
Leupetine	1	Serin-cysteine
A protein	0.0003	Saine

3.7 Western Blot

Rat kidney microsome (Upstate Biotechnology) was used as positive control for the α1 isoform of Na/K-ATPase and brain microsome for the α3. The sample was denatured by heating to 100°C for 5 min in boiling water. Then 30 – 45 μg protein of thoracic aorta and corresponding positive control (20μg microsome), as well as molecular standard

(10μg, BioRad) were loaded into wells in a BioRad mini-protean II electrophoresis cell and separated on polyacrylamide gel with Tris-Glycine-SD running buffer (in M: Tris-base-0.25, glycine-1.92 and SDS-1%). Electrophoresis lasted for about 30 min at 150 mV until the front of samples ran to the edge of the gels. The gels were removed from the glass plates and transferred to Hybond-ECL nitrocellulose membranes (Amersham Biosciences) for 2 hours at 75mV at 4°C, using a mini Trans-Blot Transfer Cell System (BioRad). The transfer buffer contains (in mM): Tris-base-50, glycine-380 and methano-20%. Then the membrane was blocked overnight at 4°C in TBS-Tween solution (in mM: Tris-base-50, NaCl-150, Tween 20-0.1%, PH 7.5) with 5% powdered non-fat milk. Next, the membrane was incubated and shacked for 60 min at room temperature with primary antibody anti-α1 (Upstate Bioechnology) mouse monoclonal IgG (1:4000 dilution), anti-α3 (Upstate Biotechnology) rabbit polyclonal antibody (1:1000 dilution) and β-actin-loading control (1:15000) (Table 3). The antibody dilutions were optimized to maximize signal and minimize background. After washing with TBS-Tween buffer for three times (3x10 min washes), the membranes were incubated for 30 min with second antibody – anti-mouse (1:2000 dilution) or anti-rabbit (1:4000 dilution) IgG antibody conjugated to horseradish peroxidase (Amersham International). The membranes were thorough washed for two times (each 10 min) and were visualized with an enhanced chemiluminescence detection kit (Amersham Biosciences) and exposed to ECL Hyperfilm (Amersham Bioscience) for 30s to 60s (β-actin for 6s). The films were then developed.

Quantification of the relative protein content was determined by scanning the blots and measuring the spot density using Alphalmager program. Because of differences in background intensity, the abundance of the detected protein was normalized to that of β-actin.

3.8 Data Analysis

Vasoconstrictor responses induced by KCl were expressed as a ratio of the contraction to KCl related to that previously obtained with phenylephrine (PhE) 1 μM (data not shown).

Table 3. Western Blot Antibodies

	Antibody	Dilution	Exposure Time (s)
Primary Antibody	α1 anti-mouse monoclonal	1:4000	50
	α3 anti-rabbit polyclonal	1:1000	120
	anti-mouse β-actin-loading control	1:15000	6
Second Antibody	anti-mouse	1:2000	
	anti-rabbit	1:4000	

Each concentration-response curve to KCl was analyzed by computer fitting to a 4-parameter logistic equation with the program prism 4.0 version (Graphpad software, San Diego, CA) using non-liner regression analysis, to evaluate the concentration producing 50% of the maximal response (EC50) and the maximum response (Emax). Data are expressed as mean experimental points with their standard error (SEM), together with the best-fitted curve to these points. Statistical significance was determined with a 2-tailed test comparing the means of independent sample groups. In K^+-induced relaxation curves, 2-way ANOVA was used for comparison of remaining contractions among groups.

To determine the protein expression of α isoforms of the Na/K-ATPase, results are expressed as relative density of protein bands in each isoform to those of β-actin on the same gel. Preliminary experiments showed that increasing the loaded protein concentrations gave proportional β-actin and α1 signals. Data were analyzed with Student's t-test for unpaired experiments. A P value of less than 5% was considered significant.

4. Results

4.1 Contractile Effect of Ouabain

Figure 14 shows contractile responses induced by ouabain in isolated aortic rings of non-pregnant and pregnant rats with or without salt supplement and obtained in normal ($[K^+]$ 5.83mM) or K^+-reduced ($[K^+]$ 2.0 mM) physiological solution. In normal physiological medium (Fig.14A), aortic ring contracted to ouabain (0.3 mM) similarly in the different groups except in non-pregnant rats with NaCl supplement. Conversely, with 1.0 mM ouabain the response was similar in all the groups, except pregnant rat on sodium supplement. This suggests that the inhibitory effect of ouabain on the sodium pump is reduced in the experimental preeclamptic model.

In the presence of K^+-reduced solution (Fig.14B), the contractile effect of ouabain on aortic ring was increased in all conditions, except in normal pregnant rats at 1.0 mM, compared to normal physiological solution (Fig.14A), but there was no statistical difference between the 2 concentrations of the inhibitor of the sodium pump. In addition, ouabain 0.1 mM induced contraction in each group (except in normal pregnant rats), which was absent in normal physiological medium. These results suggest that the pump activity is increased upon bathing aortic rings in low K^+ solution, and that pregnant animal is resistant to this increase in activity, not the preeclamptic ones.

4.2 Vasoconstrictory Response to KCl

Tables 4 and 5 report the effects of 0.9% NaCl supplement on maximal responses and sensitivity to KCl without and with ouabain 0.3 or 1.0 mM pre-contraction in aortic rings of non-pregnant and pregnant rats in normal and low K^+ physiological solution. Figure15 and 16 show the concentration-response curves to KCl. In normal physiological solution, the maximal response to KCl in the absence of ouabain is reduced in aortic rings of pregnant compared with non-pregnant rats on normal salt intake (from 1.14 ± 0.06 to

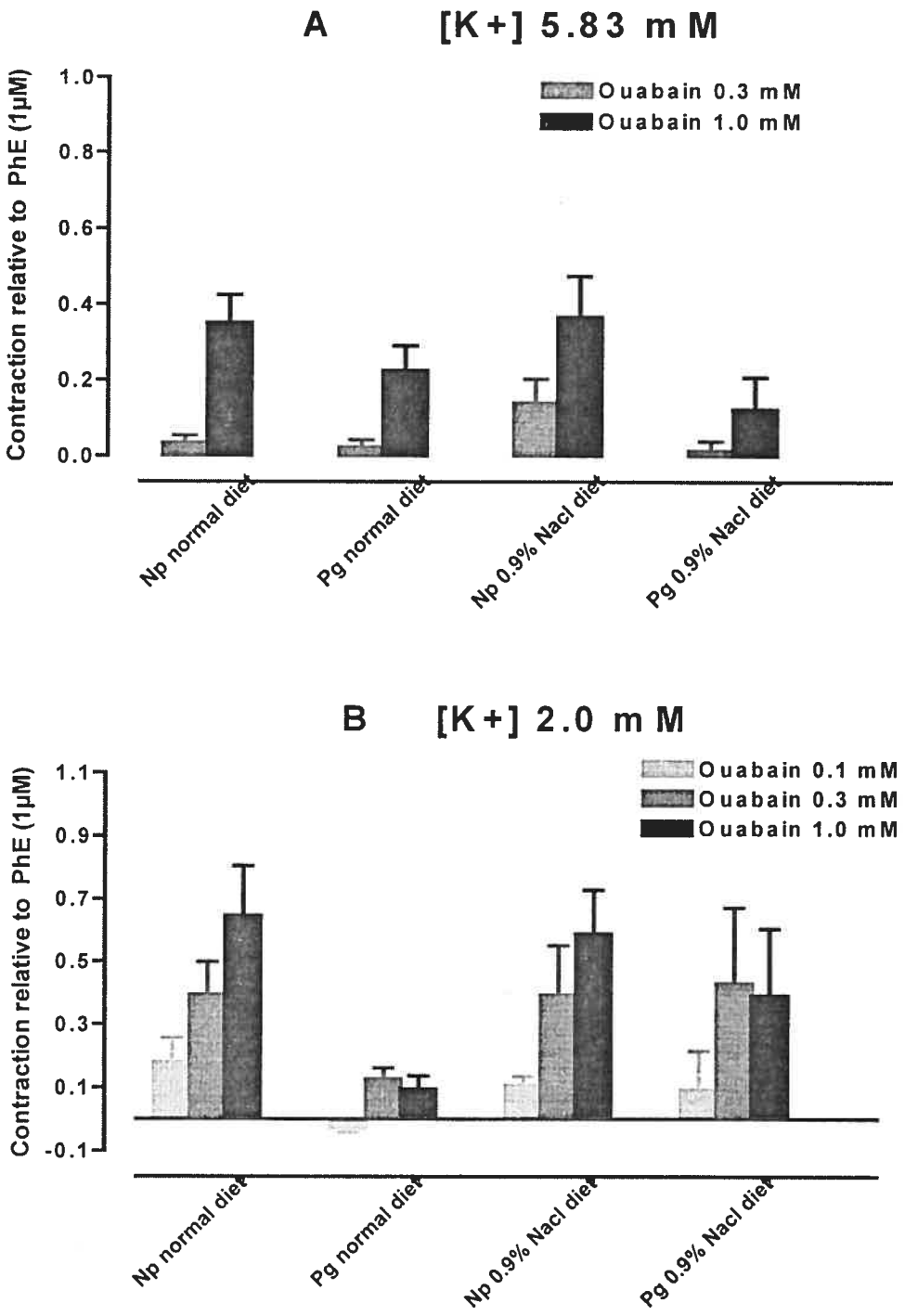


Figure 14. Contraction induced by ouabain on the aortas of non-pregnant and pregnant rats in normal and increased sodium intake. Rings were incubated in a solution containing 5.83 mM (A) or 2.0 mM (B) of $[K^+]$.

0.98 ± 0.03, P < 0.01, table 4) but no change during 0.9% Nacl supplementation. Sensitivity to KCl is also decreased (from 1.85 ± 0.06 to 1.58 ± 0.03, P< 0.01), again with no change in 0.9% NaCl intake. High salt intake in pregnant rats (experimental preeclamptic rats) did not affect maximal response or sensitivity to KCl.

Concentration-response curves to KCl that were obtained after stimulation by ouabain in non-pregnant 0.9% salt intake and normal pregnant rats shifted to the left, showing that ouabain potentiated the response to KCl in these two groups. For example, in the former group, maximal responses to KCl in the presence of both o.3 and 1.0 mM ouabain significantly increased compared with control (from 1.16 ± 0.04 to 1.40 ± 0.04, P < 0.05 and to 1.46 ± 0.05, P < 0.05, respectively, table 4), and the sensitivity to KCl is also higher in the pre-incubation of 1.0 mM ouabain (from 1.86 ± 0.04 to 2.04 ± 0.06, P < 0.05). In normal pregnant group, maximal response to KCl is statistically enhanced (from 0.98 ± 0.03 to 1.10 ± 0.03, P < 0.05), and sensitivity also increased in the presence of both 0.3 and 1.0 mM oubain (from 1.58 ± 0.03 to 1.70 ± 0.02, P < 0.05 and to 1.91 ± 0.04, P < 0.01, respectively). However, ouabain did not produce potential effect on reactivity to KCl in experimental preeclamptic rats.

These data reveal that the aortic reactivity to KCl was diminished during normal pregnancy but not in PE. Ouabain increased the vascular reactivity to KCl in non-pregnant high sodium intake rats and pregnant rats in regular diet. Experimental PE obliterated this enhanced response to KCl.

In low K^+ solution, similar to normal physiological medium, the maximal response and sensitivity to KCl are reduced in pregnant rats compared to non-pregnant rats in normal diet (from 1.24 ± 0.04 to 1.03 ± 0.02, P< 0.05, and from 1.76 ± 0.03 to 1.54 ± 0.03, P< 0.05, respectively, table 5). But in experimental preeclamptic group, there were no significant changes in maximal response or sensitivity to KCl compared to normal pregnant rats. In addition, low potassium medium also enhanced the maximal response to KCl in each group.

Table 4. Parameters of concentration-response curve to KCl in aortic rings, bathed in normal physiological solution, from non-pregnant and pregnant rats on normal and increase sodium intake.

	Basal responses	Emax	EC50
Np Ctl	-0.004 ± 0.10 (10)	1.14 ± 0.06	0.014 (1.85 ± 0.06)
oua 0.3	0.04 ± 0.08 (9)	1.11 ± 0.04	0.014 (1.85 ± 0.05)
oua 1.0	0.34 ± 0.09* (9)	1.24 ± 0.05	0.011 (1.94 ± 0.07)
Np 0.9%	-0.01 ± 0.07 (8)	1.16 ± 0.04	0.014 (1.86 ± 0.04)
oua 0.3	0.14 ± 0.07 (8)	1.40 ± 0.04*	0.011 (1.96 ± 0.03)
oua 1.0	0.36 ± 0.09* (8)	1.46 ± 0.05*	0.009 (2.04 ± 0.060*
Pg Ctl	-0.02 ± 0.03 (11)	0.98 ± 0.03•	0.026 (1.58 ± 0.03)•
oua 0.3	0.02 ± 0.04 (11)	0.99 ± 0.03	0.020 (1.70 ± 0.02)*
oua 1.0	0.22 ± 0.04* (11)	1.10 ± 0.03*	0.013 (1.91 ± 0.04)*
Pg 0.9%	0.00 ± 0.08 (6)	1.08 ± 0.07	0.024 (1.63 ± 0.09)
oua 0.3	0.02 ± 0.10 (6)	1.20 ± 0.07	0.019 (1.72 ± 0.05)
oua 1.0	0.16 ± 0.10 (6)	1.16 ± 0.08	0.020 (1.69 ± 0.07)

Values are means ± SE (n=8). Basal response is the tone left after 15 min preincubation with ouabain; EC50, in molar concentration with logEC50 in parenthesis; Emax in relative response to PhE (1uM). * vs respective control and • vs non pregnant control.

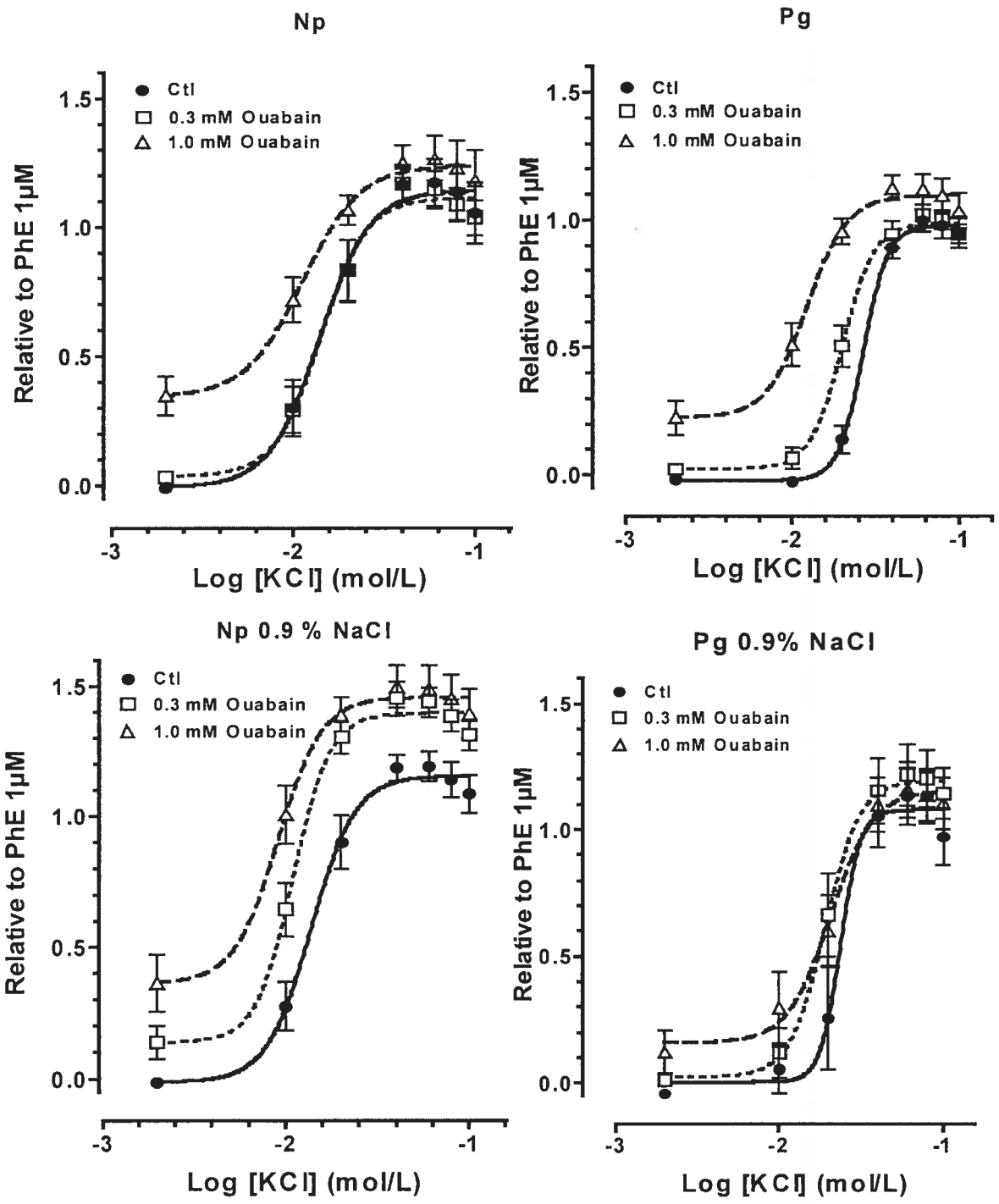


Figure 15. Concentration-response curves to KCl in aortic rings from non-pregnant and pregnant rats in normal diet and on 0.9% NaCl supplement. Rings were incubated in *normal physiological solution.*

In the presence of ouabain, concentration-response curves to KCl shifted to the left, the same situation as in normal physiological environment, demonstrating the potential function of ouabain in response to KCl in low K^+ solution, especially in sensitivity. EC50 reduced in all the groups with 1.0 mM oubain preincubation, and in normal pregnant rats, EC50 decreased with both 0.03 and 1.0 mM ouabain preincubations. As shown in table 5, in control group, sensitivity was increased from 1.76 ± 0.03 to 2.03 ± 0.10, $P< 0.01$; in non-pregnant 0.9 % NaCl group, from 1.81 ± 0.05 to 2.02 ± 0.07, $P < 0.05$; in normal pregnant rats, from 1.54 ± 0.03 to 1.67 ± 0.04, $P < 0.05$ (ouabain 0.03 mM) and to 1.76 ± 0.05, $P < 0.05$ (ouabain 1.0 mM); in experimental preeclamptic rats, from 1.54 ± 0.07 to 1.92 ± 0.11, $P <0.01$.

These results indicate that vascular response to KCl is decreased during normal pregnancy but not in PE. Ouabain potentiates this response in aortic rings of pregnant rats.

4.3 Relaxation Curves to KCl

Relaxation curves induced by KCl in K^+-free solution were measured in aortic rings of virgin and pregnant rats in the presence or absence of the three inhibitors of Na/K-ATPase, shown in figure 17 and 18. Ouabain induced a concentration dependent inhibition of relaxant effect of KCl in both pregnant and non-pregnant groups (Fig.17). Preincubation with ouabain 0.1mM signifucantly inhibited the KCl-induced relaxation and ouabain 0.03 mM and 0.01 mM partially blocked the response.

Decrease in relaxation to KCl in the presence of ouabain vs control was significantly higher in pregnant rats compared with non-pregnant group. For example, at the point of [KCl] 3 mM, in pregnant rats, the relative relaxation magnitude of the control was 1.23, and the decreased relaxation with ouabain 0.01, 0.03 and 0.1 mM preincubation were 0.48, 0.96 and 1.11, respectively. In non-pregnant group, the relative relaxation magnitude of the control was 1.46, and the reduced relaxation in the presence of ouabain 0.01, 0.03 and 0.1 mM were 0.17, 0.60 and 1.21, respectively. There was no difference of reduced relaxation with inhibition of 0.1 mM ouabain, since it almost totally blocked the

Table 5. Parameters of concentration-response curve to KCl in aortic rings, bathed in low K^+ solution (2.0 mM), from non-pregnant and pregnant rats on normal and increase sodium intake.

	Basal responses	Emax	EC50
Np Ctl	-0.004 ± 0.07 (10)	1.24 ± 0.04	0.018 (1.76 ± 0.03)
oua 0.3	0.40 ± 0.10* (9)	1.19 ± 0.07	0.015 (1.82 ± 0.09)
oua 1.0	0.65 ± 0.10* (9)	1.26 ± 0.05	0.09 (2.03 ± 0.10)*
Np 0.9%	0.01 ± 0.10 (8)	1.26 ± 0.06	0.015 (1.81 ± 0.05)
oua 0.3	0.39 ± 0.11* (8)	1.21 ± 0.06	0.012 (1.92 ± 0.08)
oua 1.0	0.59 ± 0.09* (8)	1.32 ± 0.05	0.010 (2.02 ± 0.07*
Pg Ctl	-0.02 ± 0.03 (12)	1.03 ± 0.02•	0.029 (1.54 ± 0.03)•
oua 0.3	0.15 ± 0.05* (12)	1.12 ± 0.05	0.022 (1.67 ± 0.04)*
oua 1.0	0.13 ± 0.07 (12)	1.13 ± 0.05	0.017 (1.76 ± 0.05)*
Pg 0.9%	-0.02 ± 0.09 (6)	1.10 ± 0.08	0.029 (1.54 ± 0.07)
oua 0.3	0.46 ± 0.16* (6)	1.24 ± 0.12	0.021 (1.69 ± 0.12)
oua 1.0	0.39 ± 0.14* (6)	1.27 ± 0.08	0.12 (1.92 ± 0.11)*

Values are means ± SE (n=8). Basal response is the tone left after 15 min preincubation with ouabain; EC50, in molar concentration with logEC50 in parenthesis; Emax in relative response to PhE (1uM). * vs respective control and • vs non pregnant control.

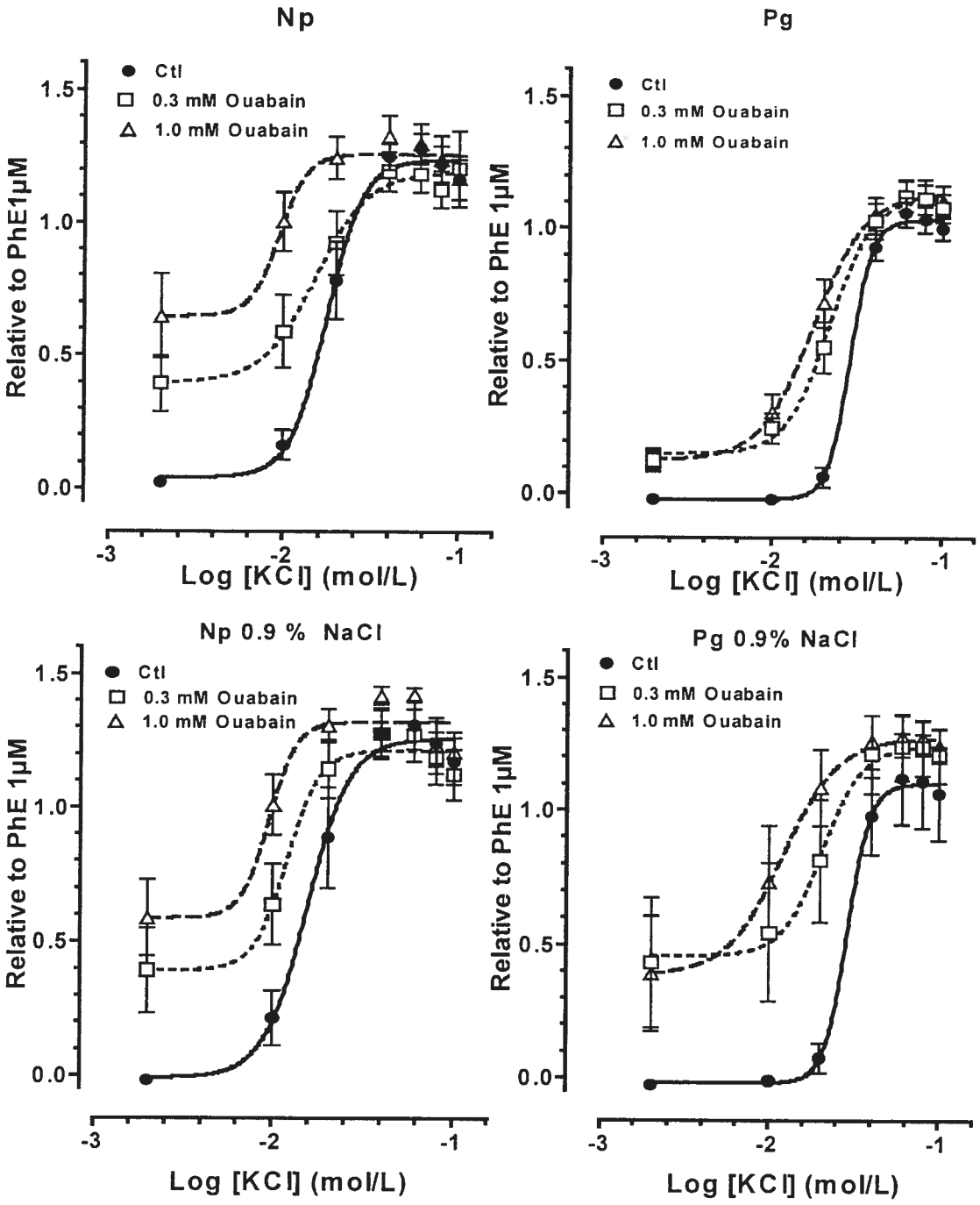


Figure 16. Concentration-response curves to KCl in aortic rings from non-pregnant and pregnant rats in normal diet and on 0.9% NaCl supplement. Rings were incubated in *a reduced potassium solution.*

KCl-induced relaxation in both groups. But the decreased relaxations in the presence of ouabain 0.01 and 0.03 mM were much less in aorta of non-pregnant than pregnant rats. These data show an increased inhibitory activity of ouabain on aortic rings of pregnant rats, suggesting Na/K-ATPase enzyme activity may be stimulated during gestation. The differences in the relaxation responses would not be affected by the magnitude of the pre-contractions induced by PhE, as the differences of pre-contractile tone were not significant between aortic rings of non-pregnant and pregnant animals.

Digoxin (0.1-1.0 μM) and MBG (0.01-0.1 μM) did not produce inhibition to K^+-induced relaxation (Fig.18), which suggests the difference in sensitivity of Na/K-ATPase during pregnancy does not seem to be associated with the presence of different isoforms of the sodium pump.

4.4 Western Blot Analysis of Na/K-ATPase α1 and α3 isoform expression

Figure 19 shows α1 isoform protein expression detected in thoracic aorta of pregnant and non-pregnant rats using monoclonal anti-α1 antibody, and figure 20 shows that in aorta of pregnant and experimental PE rats. This is close to the expected molecular mass of ~100 KDa in vascular sodium pump. Expression of α1-subunit did not change in pregnant rats compared with non-pregnant ones, but significantly increased in preeclamptic rats compared with normal pregnant ones (Table 6). The α1 is the predominant isoform detectable by Western Blot. But the α3 isoform was undetected in homogenate in the vessels studied.

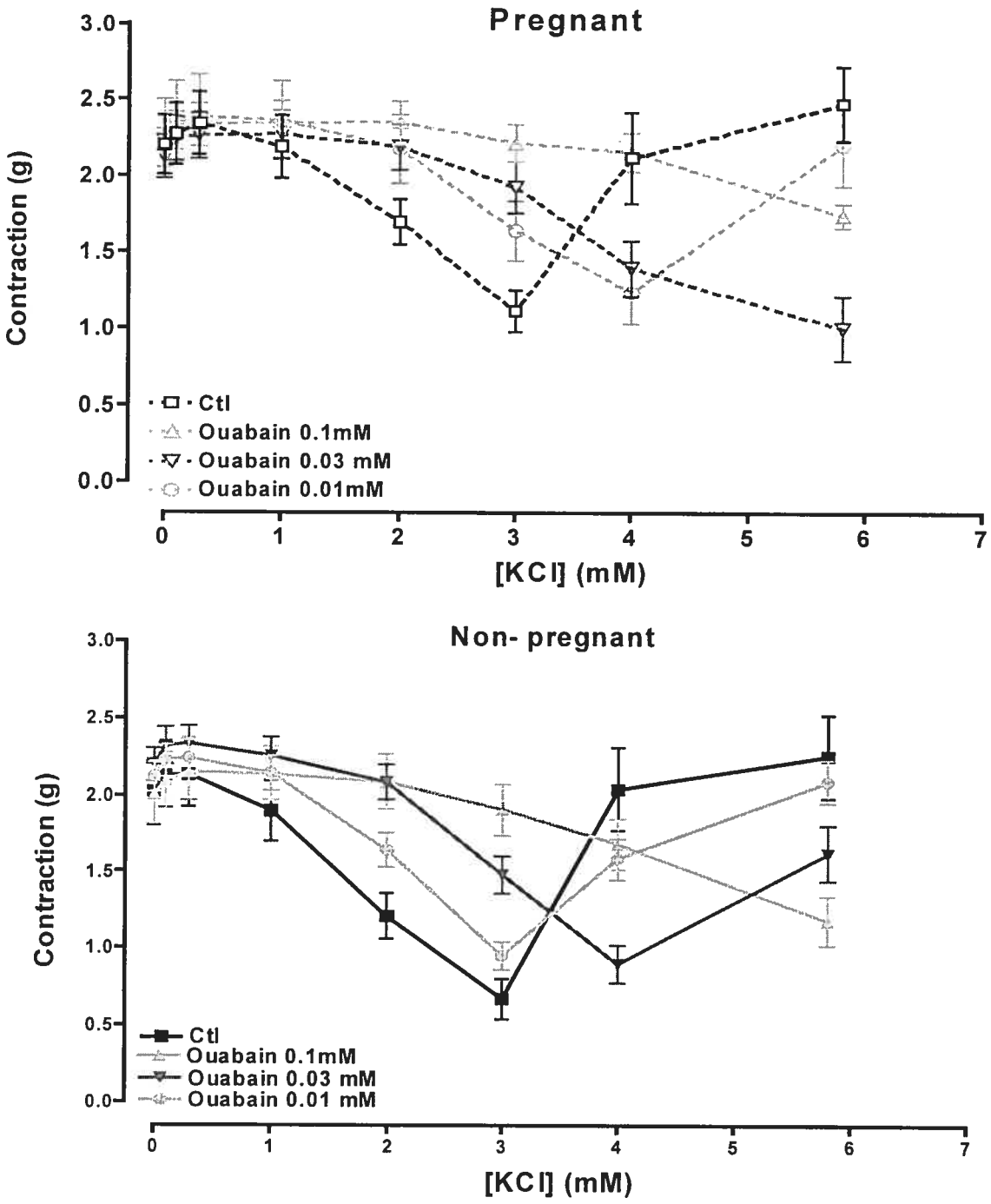


Figure 17. Ouabain induced a concentration-dependent (0.01, 0.03, 0.1mM) inhibition in KCl-induced relaxation.

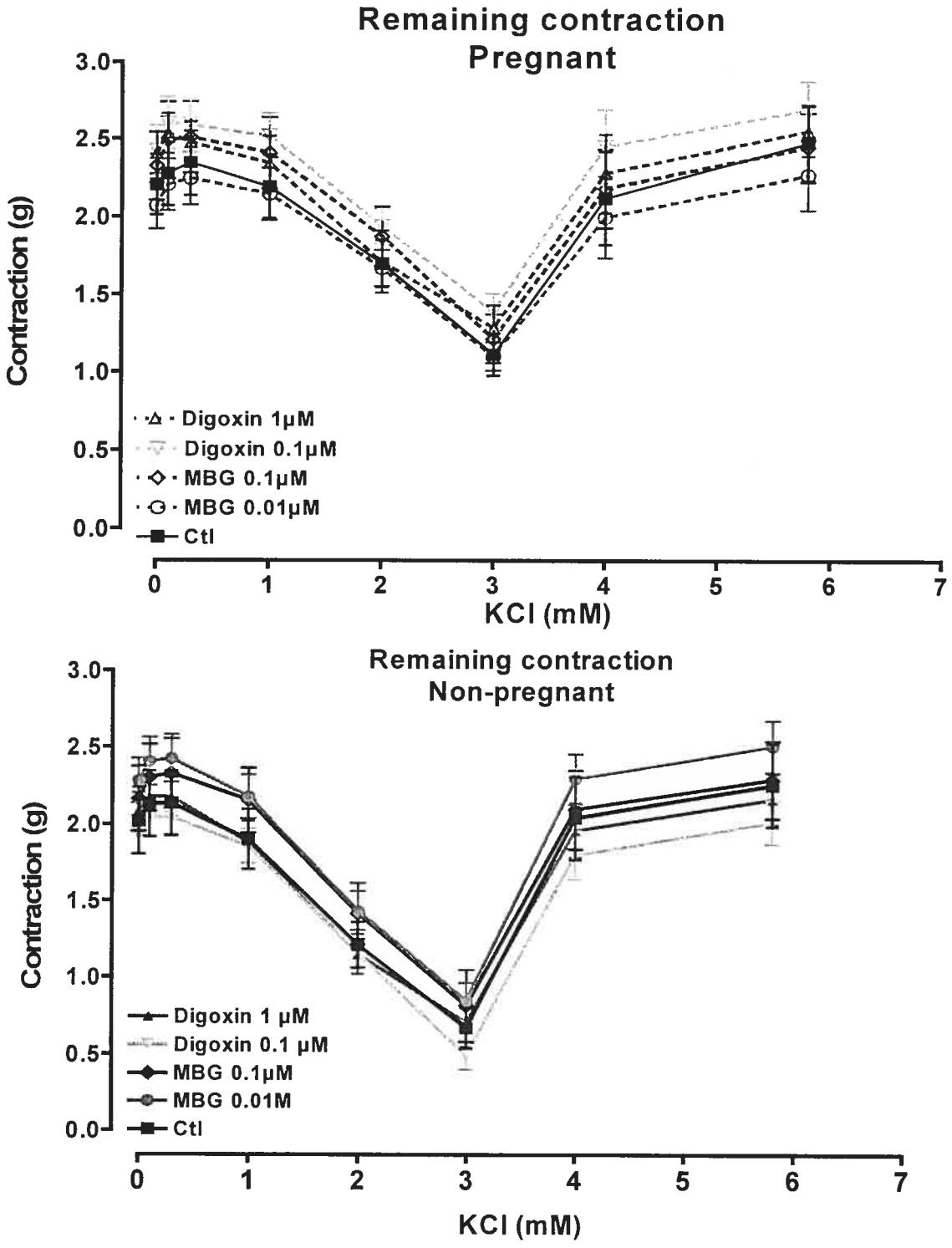


Figure 18. Digoxin (0.1-1.0 μM) and marinobufagenin (0.01- 0.1 μM) did not have any inhibitory effect on KCl-induced relaxation.

Table 6. α1 isoform Protein expression changes with pregnancy and experimental PE

Group	Mean relative signal intensity	N	P value
Pg	0.080 ± 0.015	5 X 3	
Vs			0.369
Np	0.098 ± 0.013	5 X 3	
Pg	0.306 ± 0.093	5 X 3	
Vs			0.0089
Pg + 0.9% NaCl	0.6304 ± 0.067	5 X 3	

Relative signal intensity is optical density ratio of α1 isoform to β-actin. Results are derived from 5 different pools of tissues, mach measured in triplicate.

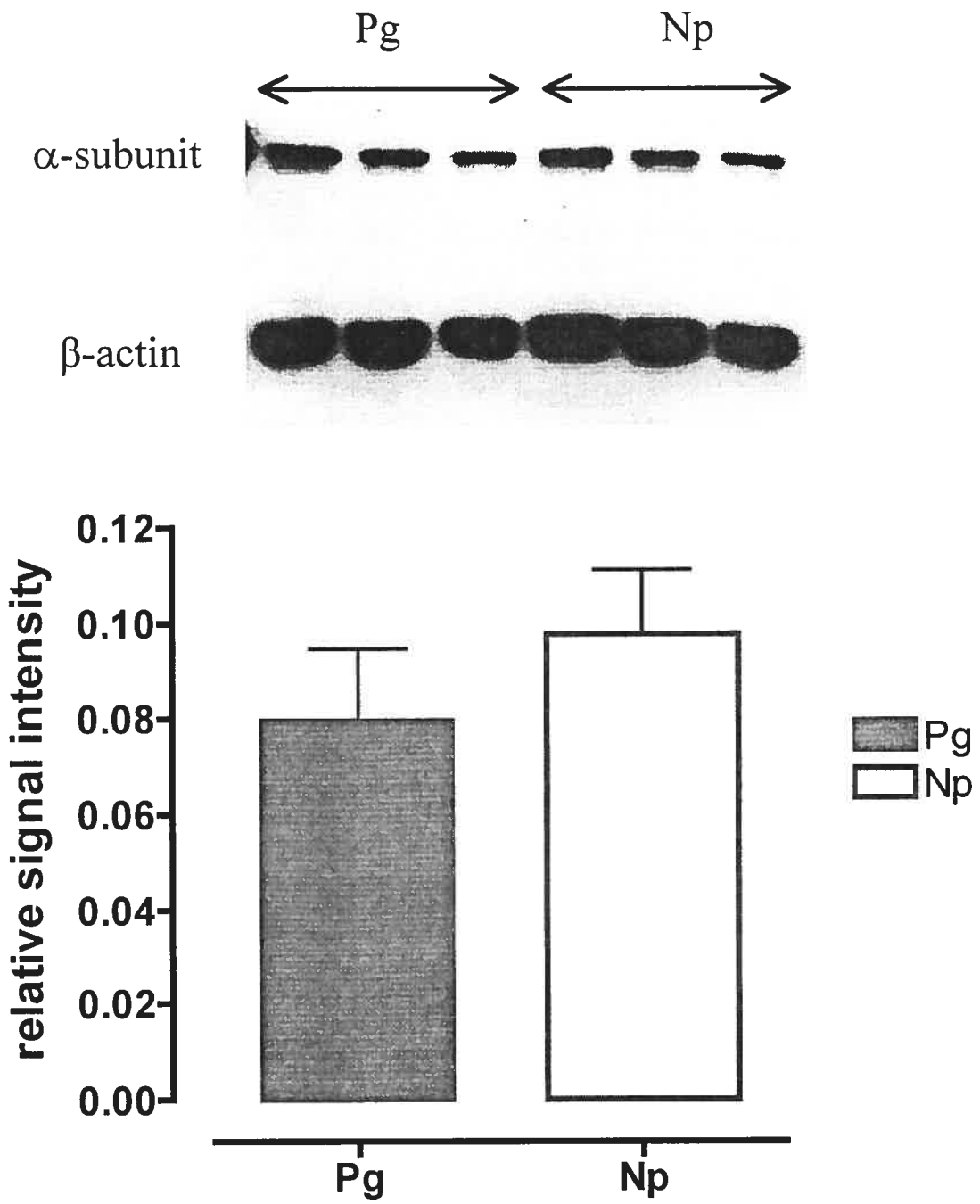


Figure 19. Expression of α1- subunit of the Na/K-ATPase by Western blot in the aortas of non-pregnant and pregnant rats .

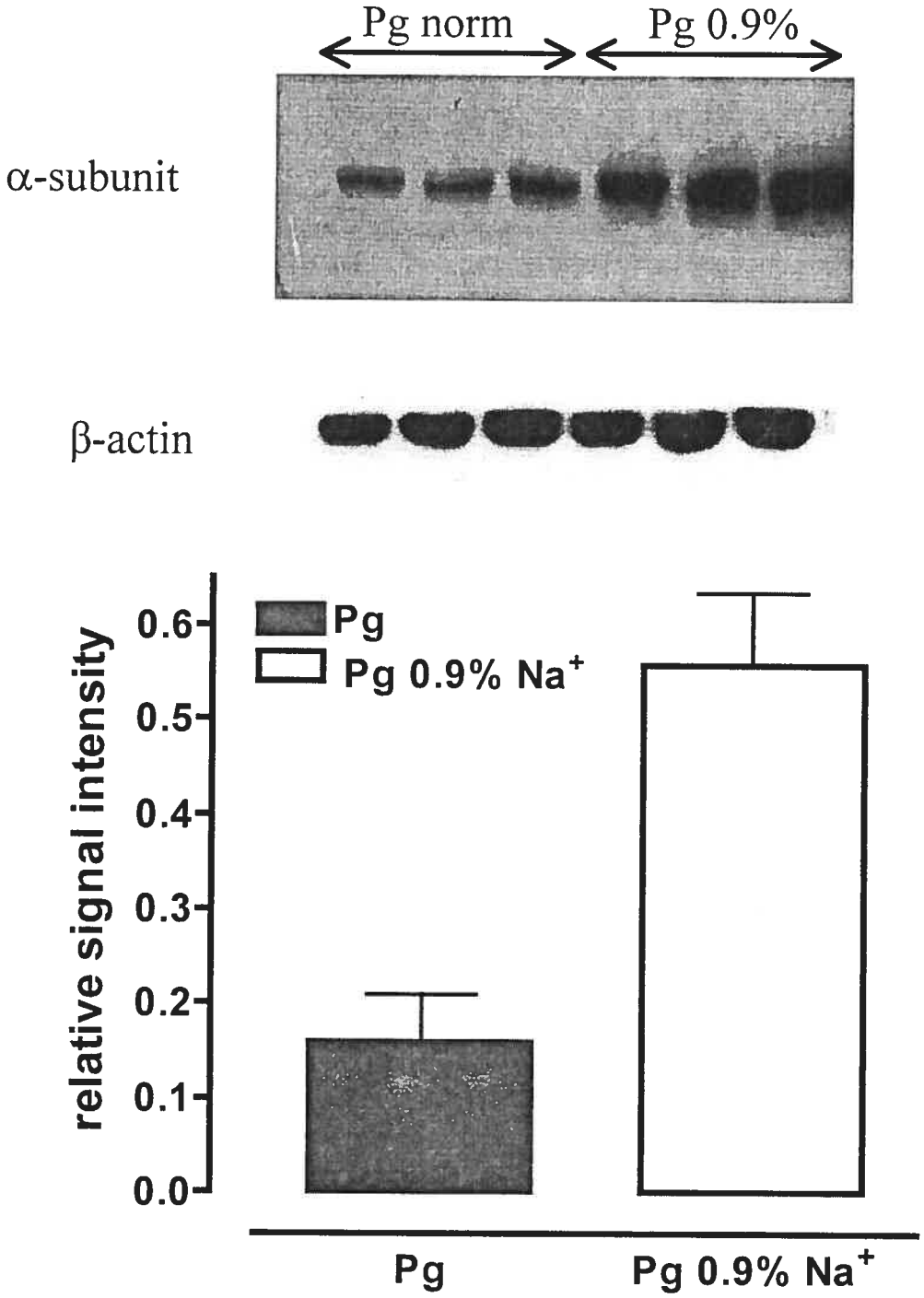


Figure 20. Expression of α1- subunit of the Na/K-ATPase by Western blot in the aortas of pregnant rats with or without sodium supplement.

5. Discussion

5.1 Overview

Pregnancy is associated with profound physiological alteration in vascular behavior, including a marked vasodilation and refractoriness to vasopressor responses. PE is a pregnancy-specific syndrome that is characterized by a marked increase in peripheral vascular resistance and in vascular tone. The mechanisms mediating the latter increased are incompletely understood. One cellular transport system affecting smooth muscle tone is the Na/K-ATPase, which plays a key role in regulating membrane potential. Reduction in its activity can result in increased muscle contraction [67]. We hypothesize that sodium pump activity and α-isoform expression in vascular smooth muscle would be modified in response to pregnancy and PE. To study any change in activity of the sodium pump in these physiological and pathological states, we measured 1) contractile effects of ouabain on isolated aortic sodium pump in normal physiological and low K^+ solutions, 2) KCl-induced relaxation in a K^+-free medium in the absence or presence of sodium pump ligands, 3) Na/K-ATPase protein expression in aorta of normal pregnant and experimental preecalmptic rats.

To establish the animal model of PE, 0.9% NaCl as drinking water was given to pregnant rats from day 15 to 22 of pregnancy, correspondent to the third week of gestation in this specie. The major findings were as following: 1) Ouabain produced contractions on isolated aortic rings. That was increased in low K^+ solution, as well as in non-pregnant rats with high salt diet. 2) Pregnancy showed refractoriness to vasoconstrictor effects of KCl, but experimental PE obliterated it. 3) Responses to KCl were increased in the presence of ouabain in all groups except experimental preeclamptic rats. 4) Relaxation to KCl was significantly reduced in aorta from pregnant compared to non-pregnant rats. Ouabain produced concentration-dependent inhibition in this relaxation, that was significantly larger in aorta of normal pregnant than in non-pregnant rats. 5) Expression of α1 subunit of sodium pump did not change in aortas of pregnant rats, but was significantly increased in aorta of preeclamptic rats.

5.2 Ouabain-Induced Contraction

Ouabain has been shown to have pro-hypertensive effect in *in vivo* studies [130-132]. Chronic administration of ouabain was accompanied by increases in blood pressure [130] and mild hypertension [131] in normotensive rats. Upon withdrawal of ouabain administration, blood pressure and plasma ouabain levels normalized within 1 week, showing a reversible hypertensive effect of ouabain [132]. In the present in vitro study, our result also showed that ouabain induced contractile responses in isolated rat aortic rings (Fig.14). This contraction after the sodium pump blockade in vascular smooth muscle can be produced through the inversion of the Na/Ca exchange, resulting in the rise in cytosolic Ca^{2+} and intracellular stores of Ca^{2+} [22, 23]. This causes the increased contractility and reactivity that underlies the increased vascular tone and peripheral vascular resistance that elevates the blood pressure.

Other cellular mechanisms involved were also reported, such as a direct effect on vascular smooth muscle mediated by depolarization and opening of the voltage-gated calcium channels (VDCC) [15, 128]. However, the contribution of VDCCs to the ouabain effect has been variable, depending on the contribution of the Na/K-ATPase to the membrane potential of the respective smooth muscle cells [22, 85]. For example, the transient contractile response induced by ouabain in umbilical arteries is sensitive to the Ca^{2+} channel blockade [129].

As shown in Fig.14a and Table 4 (Basal response), in normal physiological solution, ouabain 1.0 mM induced contraction in all the groups, but the response was smaller in experimental preeclamptic rats. This reduced inhibitory effect of ouabain on sodium pump in experimental preeclampsia suggests that aortic ring sodium pump activity is altered by high salt loading in pregnant rats. The altered activity of Na/K-ATPase results in reduced sensitivity to ouabain. Study of the modulation of the response of rat thoracic aorta to several constrictors [74] proposed that during pregnancy, sodium loading affects some mechanisms in aortic smooth muscle that are important for receptor-couple

vasoconstriction but not for depolarization-induced response. However, the exact mechanism of PE on vascular Na/K-ATPase needs to be further investigated.

In low K^+-medium (Fig.14b and Table 5 Basal response), ouabain-induced contractions were much larger than that in normal physiological solution, exceptin aorta of the normal pregnant rats. This indicates that reduced extracellular K^+ increases the inhibitory activity of ouabain on sodium pump, but pregnancy diminishes this hypertensive influence, indicating some resistance to the depolarizing effects of ouabain in aortic smooth muscle has taken place. This suggests the vascular sodium pump is activated during pregnancy. In addition, the contractile response of ouabain (1.0 mM) was more pronounced in non-pregnant rats on regular diet than in normal pregnant ones when rings were bathed in either of the two extracellular K^+ concentrations, showing a pregnancy-induced decreased response to the membrane depolarization function of ouabain.

5.3 Vasoconstrictor Activity

Vascular reactivity experiments have shown that pregnancy is often associated with blunted reactivity to many vasopressor agents [65, 66, 80-84]. This is also true in the present investigation. Table 4 and 5, and figure 15 and 16 showed that both maximal response and sensitivity to KCl were significantly reduced in aortic rings of pregnant compared to non-pregnant rats in both normal physiological and reduced K^+ solutions. It was demonstrated that the diminished response of aorta from pregnant rats to BAY K 8644, a dihydropyridine calcium channel activator, was overcome by preincubating aortic rings of pregnant rats in 10 mM KCl, suggesting that the blunted responses of blood vessels of pregnant rats could be dependent on altered membrane potential [77]. Electrophysiological data also reported that smooth muscle cells of mesenteric resistance arteries of pregnant rats were hyperpolarized by 7 mV [80]. This means that hyperpolarization of plasma membrane in blood vessels decreases sensitivity to the membrane potential depolarization effect of KCl, supporting the hypothesis that the vascular sodium pump is activated during pregnancy, since it plays a main role in resting

membrane potential regulation. Its activation during pregnancy contributes to this membrane hyperpolarization.

Of course membrane potential is largely regulated by its K^+ permeability. Which mostly depends on K^+ channels, more precisely on their open probability and on K^+ gradient across the plasma membrane. K^+ channels, when open, maintain or help to recover a basal state of polarization and modulate any depolarizing effects produced by Ca^{2+} or Na^+ influx or Cl^- efflux. Previously, we studied the effects of K^+ channel modulations on myotropic responses of aortic rings of pregnant rats and it was shown that two important K^+ channels, the ATP-sensitive K^+ channels (K_{ATP} channel) and high-conductance calcium-activated K^+ channels (Bk_{ca} channel) were involved in the blunted responses to vasoconstrictors that accompany normal pregnancy [128].

Although the K^+ gradient always has a major influence on the membrane potential, changes in the activity of the sodium pump or of other ions gradients (e.g. Na^+ gradient) can also have significant effects [119], that remain to be thoroughly investigated.

In non-pregnant animal on 0.9% NaCl supplement, only minimal change in reactivity to KCl was observed compared with non-pregnant rats in normal diet, which supports the observation [86] that phenylephrine caused a stress slightly greater in aortic strips of non-pregnant rats in high salt diet compared with those in normal diet.

In experimental PE, no significant change in response to KCl was present compared to normal pregnancy, indicating that high salt intake obliterates the decreased sensitivity to vasoconstrictor observed in normal pregnancy. This is consistent with the studies showing that sodium supplement not only prevented the end gestational decrease in blood pressure [42], but also reversed the pregnancy-associated blunting reactivity to phenylephrine, KCl and AVP [74].

To study the involvement of the sodium pump in vascular smooth muscle effects of vasoconstrictor during normal pregnancy and PE, concentration-response curves to KCl

of aortic rings preincubated with ouabain, a potent inhibitor of the electrogenic sodium pump, in normal physiological and low K^+ solutions were measured, since we expected that alteration in activity of the vascular sodium pump may cause different response to KCl during normal pregnancy and in PE.

In the normal pregnant group, the leftward shifted concentration-response curve and the decreased EC50 show that ouabain potentiates the response to KCl in aorta of pregnant compared to non-pregnant rats in both normal physiological and reduced K^+ solutions, indicating that ouabain increases the response of sodium pump induced by KCl, the voltage depend stimuli, during pregnancy. Ouabain influences the mechanism of membrane potential regulation of the aortic sodium pump more markedly, suggesting the activity of the ouabain-sensitive Na/K-ATPase is increased during this special physiological state. However, experimental PE was not associated with increased sensitivity to KCl in the presence of ouabain. It suppressed this potentiation of vasoconstriction induced by depolarization in pregnant rat rings, indicating that high salt loading may has already depolarized the plasma membrane by deactivating the Na/K-ATPase in pregnant rats. This deactivation might be achieved in two general ways: a reduction in the number of sodium pump units or an increase in level of endogenous circulating sodium pump inhibitors, which is also reported in women with PE [69]. Our Western blot result shows an increase in protein expression of the vascular sodium pump of experimental preeclamptic compared to normal pregnant rats (Fig.20). Therefore, the blunted vascular Na/K-ATPase activity of PE is not due to a reduction in the density of the pump, but a functional inhibition due to the circulating sodium pump ligands. The present observation is in accordance with the fact that endogenous sodium pump ligands are increased during pregnancy and even more in PE.

5.4 Relaxation to KCl

In this study, the inhibitory activities of ouabain, digoxin and MBG in K^+-induced relaxation of contraction produced in a K^+-free medium were measured to further

investigate the activity change of sodium pump in vascular smooth muscle during pregnancy.

According to its working principle, the Na/K-ATPase does not work in the absence of extracellular potassium ion. Omission of K^+ from the incubation medium results in almost total inhibition of the Na pump [62], which can affect intracellular Ca^{2+} and promote smooth muscle contraction in following ways: (1) The inhibition cause depolarization and increases the influx of Ca^{2+} through voltage-dependent calcium channels (VDCC) [62]. As mentioned above, membrane potential is mainly generated by the K^+ equilibrium mechanism, and VDCCs are dependent on this potential for their activity. The absence of extracellular K^+ will greatly result in a shift in the membrane potential to a less negative value, and this depolarization causes VDCCs to open. (2) Passive Na^+ leak through cell membrane during Na/K-ATPase inhibition also increases intracellular Na^+ and decreases the plasmalemmal Na^+ gradient, thus reducing the extrusion of Ca^{2+} by the Na/Ca exchange mechanism and leading to increase intracellular Ca^{2+} [3].

When K^+ is gradually returned to the organ bath, Na/K-ATPase would progressively be activated and outward current of positive charge would repolarize the cell membrane of the vascular rings [64], thereby initiating smooth muscle relaxation. As extracellular K^+ concentration is increased, the relaxation continues until extracellular potassium ion concentration reaches equilibrium with intracellular K^+, further addition of KCl will depolarize the membrane, stimulating Ca^{2+} influx and produce a reversal contraction. Therefore, the magnitude of relaxation that accompanies the introduction of K^+ is a measure of the activity of sodium pump. However, when sodium pump was blocked by its inhibitors, relaxation of aortic smooth muscle may be partially or totally inhibited. Thus, the inhibitory activities of ouabain, digoxin and MBG on the KCl-induced relaxation were determined.

Figure 17 shows significant attenuation in relaxation to KCl in aorta from pregnant compared to non-pregnant rats. This observation indicates that ouabain-sensitive Na/K-

ATPase may be deactivated during normal pregnancy. However, ouabain produced a concentration-dependent inhibition in this relaxation to KCl, which was statistically higher in aorta of pregnant than in non-pregnant rats, showing that sodium pump is activated during pregnancy. It is challenging to explain this discrepancy. KCl-induced relaxation is indicative of Na/K-ATPase activity. Other factors may affect this relaxation response in advance, as mentioned above, endogenous digitalis-like factors, which are increased during pregnancy. This substance may inhibit the sodium pump in vascular smooth muscle cells before its activation upon K^+ restoration.

5.5 Western Blot

As discussed above, the α-subunit of Na/K-ATPase contains the catalytic site for ATP and is the specific receptor for digitalic substances. It is expressed as three major isoforms that differ in turnover rates and in their tissue distribution. It's been reported that sodium pump α-subunit expression was altered in response to pregnancy and PE in critical vascular tissues [87]. The present experiment showed that expression of α1-isoform did not change in thoracic aorta of pregnant rats compared with non-pregnant ones (Fig.19), but significantly increased in preeclamptic rats compared with normal pregnant ones (Table 6, Fig.20). Recent observation reported by Fedorova et al also showed that 1.8% NaCl supplementation in pregnant rats resulted in a substantial increase in the aortic level of α1 Na/K-ATPase [133]. This shows that pregnancy has no impact on enzyme abundance of α1 isoform. The increased expression in experimental PE may be some compensatory mechanism to oppose the absence of end pregnancy decreased in blood pressure in this animal model. The expression of α-subunit is also subject to regulation by various hormones [88] and altered during experimentally induce pathological conditions [63, 89]. For example, rat models of pressure-overload cardiac hypertrophy caused no significant change in the expression of α1, but that α2 expression was repressed [10, 90, 91]. It is not known if cardiac glycosides or endogenous ouabain or experimental PE can cause these changes in rat aorta.

In the present experiment of α1 isoform expression detection, non-pregnant animals with 0.9% sodium supplement were not necessarily used as the control of experimental PE. According to the previous observation [42], 0.9% Na^+ is a low salt loading and did not affect systolic blood pressure in non-pregnant rats compared with non-supplemented control, showing that non-pregnant rats were able to maintain homeostasis in response to 0.9% sodium supplement [42]. Although often provided, non-pregnant animal on high sodium intake are not absolutely required, since the control condition for PE is normal pregnancy.

The undetectable levels of the α3 isoform in our aorta may not be surprising. Vascular smooth muscle of rats frequently has much more α1 than α3 [70]. Low α3 subunit may reflect decreased α3 production transport or membrane insertion, or increased turnover [87].

Our results indicate that change in the inhibitory activity of ouabain is associated with the change in expression of the sodium pump. Previous study in our lab reported that plasma electrolytic salt did not change in pregnant animals with 0.9% sodium supplement [42], indicating that moderate increase in sodium intake is not likely to result in higher intracellular sodium, nor the circulating ouabain-like compounds (Fig.21) and Na/K-ATPase activity. Excessive sodium loading, say 4% Na^+ and a longer duration, may probably leads to the rise in the sodium activity.

To better understand the subject in general and our experiment findings, a recapitulative schema about the relationship among PE, ouabain inhibitory activity and sodium pump is present bellowed (Fig.22).

5.6 Summary of Experiments

The present study attempts to investigate the inhibitory effect of the sodium pump ligands and protein expression of the α subunit of the Na/K-ATPase in isolated aorta of normal pregnant and experimental preeclamptic rats, and determine the change in activity of

Na/K-ATPase during normal pregnancy and PE. According to our experimental results, we can come to the following conclusions: 1) Inhibitory activity of ouabain in aortic rings of pregnant rats is increased; 2) Experimental PE reduces the inhibitory effect of ouabain; 3) Activation of sodium pump during pregnancy favor vascular smooth muscle hyperporization; 4) Increased protein expression of α1 isoform may be a biological compensational effect.

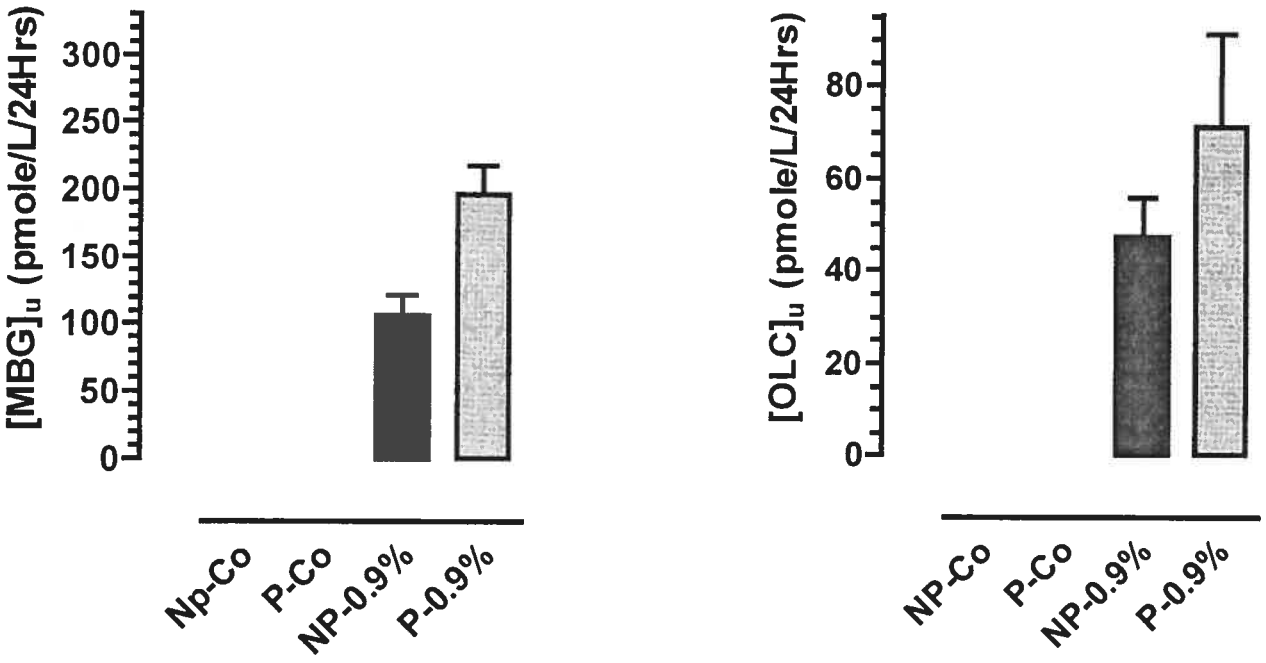


Figure 21. Immunoassay of marinobufagenine (MBG) and ouabain like compound (OLC) in urine for 24 hours (St-Louis and Bagrov, 2002 unpublished). NP-Co, non-pregnant normal diet. P-Co, pregnant normal diet.

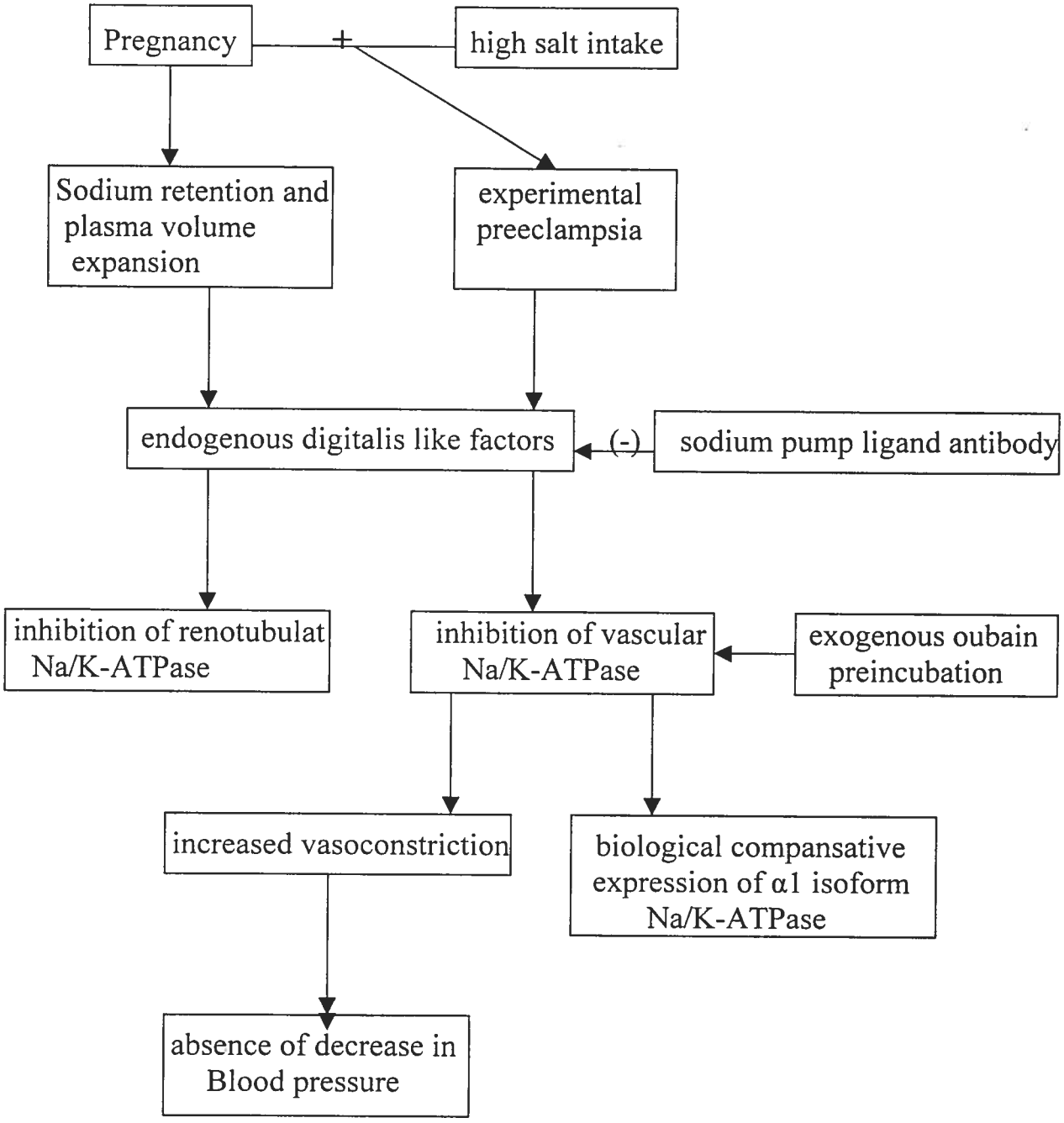


Figure 22. Scheme of relationship among PE, ouabain inhibition and sodium pump activity & expression. (-), inhibitory effect.

5.7 Future Study

Although the involvement of the Na/K-ATPase in vascular smooth muscle effects of vasoconstrictors has long been recognized, its role in vascular tone regulation and myotropic reactivity in response to pregnancy and PE has only been intensified in recent times. It's also an emerging field in cell biology and many questions need to be answer to understand the exact linkage between the inhibitory effect of Na/K-ATPase and the change in the number of sodium pump units, and the mechanism implicated in the alteration of sodium pump activity induced by high salt intake during pregnancy. Also we need to know the regulation of expression of the vascular sodium pump: how they assemble and traffic to reach its specific localization at the cell membrane during pregnancy and in the pathological state of PE.

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